THE JOURNAL

OF

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THIS VOLUME IS DEDICATED TO
PROFESSOR CAMILLO GOLGI

VOLUME 30

DECEMBER, 1918–AUGUST, 1919

PHILADELPHIA, PA.

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

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TO

CAMILLO GOLGI

PROFESSOR OF PATHOLOGY AT THE UNIVERSITY OF PAVIA, SAVANT AND CITIZEN, GUARDIAN OF PUBLIC HEALTH AND STUDENT OF HISTOLOGY, TO WHOM THE WORLD IS INDEBTED FOR A METHOD WHICH GAVE A DEEPER INSIGHT INTO THE ARCHITECTURE OF THE CENTRAL NERVOUS SYSTEM—THIS VOLUME OF THE JOURNAL OF COMPARATIVE NEUROLOGY IS DEDICATED AS A TOKEN OF HIGH ESTEEM FOR THE SCIENTIST AND THE MAN WHO, HONORED AND FULL OF YEARS, NOW WITHDRAWS FROM HIS PROFESSORIAL RESPONSIBILITIES

THE JOURNAL OF COMPARATIVE NEUROLOGY, VOL. 30, NO. 1
DECEMBER, 1918

Resumido por el autor, Olof Larsell.

Estudios sobre el nervio terminal. Mamíferos.

El autor describe con algún detalle el nervio terminal en el gato, buey y mulo, dando también descripciones más breves de dicho nervio en el caballo, perro, ardilla y en el hombre. En el buey, mulo y ardilla se describe este nervio por primera vez. En los mamiferos está formado principalmente por fibras del simpático; las del fascículo principal del nervio tienen con las que se distribuyen periféricamente una relación semejante a la que existe entre las fibras preganglionares y postganglionares. Esta semejanza está aumentada por la estructura de los racimos ganglionares y por la presencia de cestas pericelulares en muchas de las células ganglionares. También existen redes intercelulares y células típicas del simpático. Por la arteria cerebral anterior y sus ramas y por el plexo vascular del tabique nasal se distribuyen fascículos de fibras mielínicas y amielínicas procedentes del tronco principal y ganglios. Estas fibras terminan en las paredes de los vasos sanguíneos por medio de terminaciones nerviosas sensitivas y motrices. Hay algunas pruebas de que las terminaciones nerviosas libres en el epitelio nasal están relacionadas con el nervio terminal, pero la presencia indudable de fibras del trigémino en el plexo del tabique, junto con las procedentes del terminal, no permite una afirmación rotunda sin previo trabajo experimental. Estas terminaciones, junto con las de ciertas células ganglionares parecen indicar la existencia de un componente sensitivo en el nervio, distinto de las fibras aferentes del simpático. Parece claro que la inervación del órgano de Jacobson por parte del nervio terminal es incidental y secundaria. El autor expone la posibilidad de que el nervio terminal represente una división del sistema del simpático, relacionada con el cerebro anterior.

Translation by Dr. José Nonidez,
Columbia University

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 9

STUDIES ON THE NERVUS TERMINALIS: MAMMALS[1]

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FORTY-NINE FIGURES

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I. INTRODUCTION

Discovery and naming. The cerebral nerve now known as the nervus terminalis first began to attract the attention of morphologists in 1894. In that year Pinkus described in the dipnoan fish, Protopterus, a hitherto unrecognized nerve of the forebrain.

[1] Contribution from the Zoological Laboratory of Northwestern University, William A. Locy, Director.

This nerve had previously been figured by Fritsch in the selachian, Galeus, in 1878, and had been mentioned in 1893, by C. L. Herrick in the urodele amphibian, Necturus. The observations of Fritsch and Herrick, however, were merely incidental, in connection with other work, and the significance of the observations escaped them.

After these anticipatory glimpses the nerve remained unnoticed until Pinkus described it in Protopterus in 1894. In 1895, in a more extended paper, Pinkus figured and described it as lying ventral to the olfactory nerve and extending caudad over the ventral surface of the forebrain to the recessus praeopticus, its peripheral terminations being in the olfactory sac. Thus, although first figured in selachians, it was first described with sketches, in the Dipnoi.

Shortly afterward, Allis ('97) described and figured in the ganoid fish, Amia, a strand which he traced centrally to the bulbus olfactorius and peripherally to the olfactory capsule. He considered this strand to be homologous with the nerve described by Pinkus. In his comments regarding the possible function of the nerve, he suggested that it might be of sympathetic type. This is interesting in view of the position taken by Brookover and others and of the demonstration of the presence of sympathetic fibers in the bundle of the nervus terminalis of mammals.

Now appeared the first study of the embryological history of the nerve (Locy, '99) together with a description of its adult condition in Squalus acanthias. In this form the nerve was described and figured as possessing a compact ganglion, as connected with the brain in the fissure between the lobes of the telencephalon, and as distributed anteriorly to the lateral part of the olfactory capsule and entering between the folds of the nasal epithelium. It was claimed that the nerve arises from the neural crest before the appearance of the olfactory fibers. At that time Locy considered as doubtful its homology with the nerve described by Pinkus, and provisionally designated it as a median 'accessory olfactory strand.' In 1903, after observing the same nerve in six genera of selachians, Locy reversed his earlier opin-

ion and concluded that the nerve of selachians is homologous with that described by Pinkus in Protopterus and later by Allis in Amia. The same author in his paper of 1905, based on the examination of twenty-seven species of adult selachians and the embryological history of the nerve in Squalus, proposed the name of 'nervus terminalis' for this new nerve. This name has been generally adopted.

In the interval Sewertzoff ('02) had described the nerve in embryos of Ceratodus, finding it ganglionated and terminating peripherally in the mucous membrane of the anterior nasal chamber—not in the sensory epithelium. On account of its point of central connection with the brain he suggested for it the name of 'nervus praeopticus.' This nerve had also been cited in the adult of Ceratodus by K. Fürbringer in 1904, as well as by Bing and Burckhardt in 1904 and 1905.

Conditions in the different classes of vertebrates. Since 1905 a considerable literature has accumulated regarding the nervus terminalis. Its presence has been demonstrated in all cases of vertebrates except the cyclostomes (and possibly the birds) and various suggestions regarding its function have been made from time to time.

Inasmuch as the present paper deals with the nervus terminalis chiefly in mammals, it is not necessary to enter into a review of the rather extensive literature of the nerve in the lower vertebrates, But, since the nerve presents some modifications and some differences in the mammals, it is advantageous both for comparison and for discussion of results, to have a brief statement of the chief structural features which have been observed in other classes of the phylum. The nervus terminalis appears to be more generalized in the fishes, especially in the selachians, and in discussing its relations in mammals it would be a mistake to disregard the findings in the lower vertebrates.

Fishes: a) Selachians. In a paper on the telencephalon of the selachians, Johnston ('11) shows that typically the nervus terminalis enters the brain substance near the recessus neuroporicus internus. He remarks: "Some evidence has appeared recently (Burckhardt, '07, p. 340; Brookover, '10) that the nervus ter-

minalis is a mixed nerve, containing in some fishes peripheral sympathetic fibers distributed to the blood-vessels. These efferent fibers make their exit in a dorsal root (N. terminalis) as the viscero-motor fibers typically do in the spinal region of lower vertebrates."

Belogolowy ('12), from a study of young selachian embryos, concludes that the nervus terminalis is derived from the terminal portion of the neural crest. This was also claimed by Locy ('99) and ('05 a).

McKibben ('14) studied the histological structure of the ganglion terminale of Mustelus by intravitam methylene-blue staining. He found the great majority of the cells to be multipolar and "few if any bipolar cells." Landacre ('16), from observations on Squalus embryos, holds that the terminalis is combined with the olfactorius as a possible general cutaneous component of the latter.

b) *Ganoids.* Through the studies of Brookover ('08 and '10) we have very complete reports of the terminalis for the ganoid fish Amia. He concludes that the ganglion of the terminalis arises from the olfactory placode a little later than do the fibers of the olfactory nerve. He doubts its independence and is disposed to consider it a part of the olfactory system. In histological observations he finds ganglion cells chiefly of sympathetic type. He also finds fibers along the blood-vessels and a connection between the terminalis fibers and the ciliary ganglion, and suggests that the circumstantial evidence leads one to ascribe to it a vasomotor function, in part. The same author gets similar results from Lepidosteus ('14) where the ganglion terminale appears to arise from the olfactory placode after the formation of the olfactory fibers. "The disposition of the cells in Lepidosteus in a more compact central and a diffuse peripheral ganglion allows of its falling quite naturally into the morphological relations of the typical autonomic system."

This shows the tendency toward interpreting the terminalis as sympathetic in nature (or at least as containing sympathetic fibers) which becomes more marked in studies of the mammals.

c) Teleosts. The presence of the nervus terminalis in bony fishes was first reported by Sheldon and Brookover ('09) in the carp (Cyprinus carpio). Sheldon ('09) independently takes up the central course of the nerve. The tract is composed of unmyelinated fibers. Numerous scattered ganglion cells were found on the ventromedial aspect of the olfactory nerve, from some of which coarse fibers were traced to the olfactory epithelium where they were distributed with the olfactory nerve fibers. Centrally the fibers for the most part decussate at the anterior commissure, but no exact nuclear connection could be found.

Brookover and Jackson ('11) studied the development of the terminalis in Ameiurus, and also its adult relations by means of the silver-impregnation methods. They find the nerve to be closely related in its development to the olfactory nerve and are inclined to consider it a part of this rather than as an independent nerve. They point out the proximity of fibers of the nervus terminalis to blood-vessels, but find only a single instance where the blood-vessel definitely appeared to be innervated by terminalis fibers. A vasomotor functon is suggested.

Amphibia. C. Judson Herrick ('09) found in larval and adult frogs a bundle of unmyelinated fibers corresponding so closely to the nervus terminalis of the fishes in its central course that he considered it to be homologous with the latter. He was unable from his material to determine peripheral terminations. The nerve is not exposed as in selachians. It runs along the ventral border of the olfactory nerve and becomes imbedded in the brain substance just caudad to the glomerular formation. Within the brain substance it passes caudally (in one case showing arborizations in the lamina terminalis) and the fibers cross in the middle part of the anterior commissure.

McKibben ('11) traces the course of the nervus terminalis in Necturus and a number of other tailed amphibians. As in the frog, it is mainly imbedded in the brain substance. The principal central distribution is to the preoptic nucleus. The central bundle undergoes a partial decussation in the anterior commissure, but groups of direct fibers extend further backward from this point, giving off branches at intervals. The continuity of

these fibers with those of the nervus terminalis was not clearly demonstrated. The fiber groups were lost in the brain substance without evidence of definite terminations, though the fascicles reach backward into the mesencephalon (hypothalamus and interpeduncular region), a condition not yet noted in other forms. Peripherally are ganglion cells with fibers going apparently to the nasal capsules.

C. Judson Herrick ('17), in connection with other studies, has completely confirmed in Necturus the findings of McKibben.

Reptilia. Among reptiles the nervus terminalis has been found by Johnston ('13). In a paper, which embraces also a consideration of the nerve in pig, sheep, and human embryos, he describes the terminalis in embryos of the turtle (Emys lutaria). In Emys the nerve emerges from the rostral end of the median wall of the brain hemisphere, caudal to the olfactory bulb. From this point "It descends over the medial surface of the bulb and olfactory nerve and bears clumps of ganglion cells at several points of its course. It comes into close relation with the dorsal division of the olfactory nerve, but is distinguished from it." Peripherally the fibers of the nervus terminalis are distributed with those of the dorsal division of the olfactory nerve to the extreme lateral portion of the nasal sac, which is interpreted by Johnston to correspond with the vomeronasal organ of mammals.

According to McCotter ('17), the dorsal division of the olfactory nerve of the turtle is to be considered homologous with the vomeronasal nerve of mammals, and the dorsal portion of the formatio olfactoria of the bulb, as illustrated in Johnston's figure 11, corresponds to the accessory olfactory bulb of mammals.

Birds. Whether or not the ganglion cells observed by Rubaschin ('03) in the chick embryo represent cells of the nervus terminalis is problematical. This writer describes a ganglionic mass related on the one hand to the trigeminus nerve and on the other to the olfactory mucosa. Axones from this mass were traced to the Gasserian ganglion. Two types of cells were found in the ganglionic knots: 1) bipolar cells resembling those of the intervertebral ganglia, and 2) multipolar cells with numerous proc-

esses, of which one in each case enters the 'ramus olfactorius nervus trigemini.' Cells of this type are relatively few in number. These observations have been interpreted by some writers to indicate the presence of the nervus terminalis in birds.

Mammals. Since 1905 the nervus terminalis of mammals has been dealt with in no less than nine scientific memoirs. In some cases it has been confused with the fibers of the vomeronasal nerve (Devries, '05; Döllken, '09), but in most cases the fibers of the terminalis are distinguished from those of the vomeronasal. Notwithstanding these investigations, the nervus terminalis in the mammals is very imperfctly known and its relations are obscure.

The first published notice of this nerve in the mammals was made by DeVries in 1905. He found in the human fetus of three to four months a transitory ganglion which he regarded as corresponding to the ganglion of the nervus terminalis, and which he designated 'ganglion vomero-nasale.' He also found similar conditions in the guinea-pig. His assumption that the vomeronasal nerve of mammals represents the nervus terminalis of selachians and other fishes is not substantiated by more recent work.

Döllken ('09) studied embryonic stages of rabbit, mouse, guinea-pig, pig, and human. His account of the central connections of what he describes as the nervus terminalis is extended. He finds roots which enter the brain and reach the cortex, the gyrus fornicatus, the hippocampus, and the septum pellucidum. The peripheral distribution he describes as being by four or five strands to the vomeronasal organ. It seems clear from his description and figures that he is dealing almost entirely, if not completely, with the vomeronasal nerve, which Read ('08) and McCotter ('12) have clearly differentiated from the olfactory fibers proper in mammals, and McCotter ('17) in the turtle and the frog.

In a paper already referred to in connection with the nervus terminalis in reptiles, Johnston ('13) also describes the nerve in embryos of pig, sheep, and human. In pig embryos he finds the root of the terminalis entering the brain at the ventral end of the fissura prima. The fibers are traceable for some distance within

the brain toward the anterior commissure. The peripheral course of the nerve is described as being in the wall of the septum nasale, along which it passes by several strands to the wall of Jacobson's organ and to a small area of the nasal sac immediately adjacent.

In the human embryo Johnston found essentially the same central relations as in the pig, but the peripheral distribution is by a network of nerve bundles in the nasal septum.

He concludes that "the evidence at present in hand seems to establish beyond doubt the presence in all vertebrates of a receptive component in the nervus terminalis supplying ectodermal territory. This component is derived either from the terminal part of the neural crest (Johnston, '09 b; Belogolowy, '12) or from the olfactory placode (Brookover, '10). The nerve is distributed to the nasal mucosa, or to a specialized part of it, the vomeronasal organ."

McCotter ('13) demonstrated by dissection the main central bundle of the terminalis in the adult dog and cat. He also found the typical ganglion cells of the nerve distributed along the vomeronasal strands. No differentiation of fibers from those of the vomeronasal nerve was obtained by the staining methods used.

The application to the problem of a modified pyridin-silver technique by Huber and Guild ('13), served to clearly differentiate the terminalis from the vomeronasal nerve. These investigators used rabbit fetuses and young rabbits. They were fortunate in securing a differential stain which made it possible to follow the fibers of the two sets of nerves individually. They conclude that

> this nerve is not a component of the olfactory and vomero-nasal complex, but an independent nerve, with central connections by means of several small roots to the ventro-mesial portion of the forebrain, caudal to and independent of the olfactory stalk, and courses in the form of a loose plexus along the ventro-mesial surface of the olfactory bulb, reaching the nasal septum and the mesial surface of the vomero-nasal nerve, which nerve it follows to the vomero-nasal organ, and is further distributed to the septal mucosa anterior to the path of the vomero-nasal nerve, in which region especially it is joined by terminal branches of the trigeminus, mainly from the naso-palatine bundles.

Numerous ganglionic masses of various sizes are found. One group of relatively large size located near the most caudad bundle of the vomeronasal nerve a short distance from where the latter leaves the accessory olfactory bulb is regarded as the ganglion terminale of authors. These groups of ganglion cells present the appearance of small sympathetic ganglia, and the authors state that the nerve fibers have more the appearance of sympathetic and preganglionic fibers than of neuraxes and dendrites of sensory neurones. A comparison of these cells with the cells of the Gasserian ganglion of the same animals revealed the fact that the terminalis cells are of smaller size.

Distribution of terminalis fibers to blood-vessels and septal mucosa was considered probable from the observations, but the authors hesitated to assert such distribution because of the commingling of fibers from the trigeminus with those of the nervus terminalis.

Johnston ('14) describes the central relations of the terminalis in the adult human, in the horse, porpoise, and the sheep. Numerous rootlets were found in some, especially in the horse, while in other forms only two or three are enumerated. In the horse, a large, compact ganglion is described. In the other mammals the ganglionic masses are described as being smaller but more numerous, and more or less scattered along that portion of the nerve which it was possible to examine.

Brookover ('14) independently of Johnston discovered the central portion of the nerve in the brain of the adult human, and reached substantially the same conclusions as to central connections as did Johnston, namely, that its intracranial course lies over the middle of the gyrus rectus and appears to enter the brain substance in the region of the medial olfactory striae.

Both central and peripheral distribution in the human fetus is described by McCotter ('15). He indicates the peripheral course in the nasal mucosa, where it resembles in general the conditions found by Huber and Guild in the rabbit. Centrally the majority of the fibers form a single strand.

The latest paper which has come to notice has appeared since the greater part of the observations recorded in the present ar-

ticle were made. In this paper Brookover ('17) describes the peripheral distribution of the terminalis in the nasal septum of the human fetus at full term. This material was prepared by the pyridin-silver method.

A large plexus of fibers is found anastomosing over the nasal septum deep to the main arteries. The writer states that this network "is so large that it may be considered as hypertrophied as compared to the known development in other mammals, without apparently increasing the central root." There are indications of a sympathetic chain connection with the sphenopalatine nerve and ganglion.

It seems clear that in selachians, Dipnoi, ganoids, teleosts, Amphibia, reptiles (turtle at least), and mammals there is common to all a nerve with central connections with the brain near the embryonic anterior neuropore, and having a primary peripheral distribution to some part of the lining of the nasal cavity.

II. DESCRIPTIVE PART

Material and methods. The original design of this investigation was to make a comprehensive analysis of the nervus terminalis in the various classes of vertebrates. More difficulties of analysis and interpretation are encountered in mammals than in the other groups, so that the present contribution is limited in its scope to the conditions found in certain mammals, with the expectation of extending these observations in a subsequent paper to other classes.

The studies were carried on in the Zoological Laboratory of Northwestern University from 1915 to 1918, inclusive, under the direction of Prof. William A. Locy, to whom I express my sense of indebtedness for helpful advice and criticism. My thanks are also due Prof. S. W. Ranson, of Northwestern University Medical School, for valuable suggestions.

The mammalian material used consists of the following:

1 longitudinal and 1 transverse series of 10 mm. kitten embryos, fixed in 10 per cent formalin and stained with haematoxylin.

1 sagittal series through forebrain and nasal septum of kitten one day old, treated by the pyridin-silver process.

1 sagittal series through forebrain and nasal septum of kitten two weeks old, pyridin-silver method.
2 sagittal series of nasal septum of kittens two weeks old, pyridin-silver method.
1 sagittal series through forebrain and nasal septum of kitten one day old, fixed and decalcified in Zenker's fluid and stained by the Weigert method.
2 septal mucosae of kitten one day old, stained with methylene-blue.
Numerous series of pig embryos at various stages, stained with haematoxylin or with Mallory's connective tissue stain.

In addition to these sections, the following materials were also studied according to the method indicated: Numerous dissections of kittens, of puppies, and of adult dogs and cats were made. The method described by McCotter, by which the head is fixed in Müller's fluid to which acetic acid has been added, was used with good results. Fixation in 10 per cent formalin, followed by decalcification in 10 per cent nitric acid, was also adopted in many instances. A light surface stain with borax-carmine was found advantageous in differentiating the nervus terminalis from the neighboring tissues.

Frozen beef brains were obtained from the Chicago Stock Yards, and were found to be very favorable for the dissection of the delicate strands of the nervus terminalis. These refrigerated brains were allowed to thaw in a solution of 10 per cent formalin at room temperature. Besides being relatively easy to dissect, this material responded well to the gold-chloride technique and gave excellent histologic preparations. A number of beef fetuses of 110 mm. to 140 mm. greatest length were also dissected. These had been preserved in formalin. Supplementary studies were made on beef material from which the meninges and a portion of the brain beneath the region of the nervus terminalis were removed and fixed in 1 per cent osmic acid, immediately after the brain was taken from the cranial cavity. Most of the material thus obtained was still warm when fixed. It was made possible to obtain this through the courtesy of Swift & Company.

The brain of a full-term mule, freshly removed and fixed in 10 per cent formalin, was obtained. Another mule fetus of 121 mm. greatest length was also studied. The brain, in situ, and the nasal septum of an adult horse was obtained through the

courtesy of the Chicago Veterinary College. This was fixed in 10 per cent formalin and decalcified in nitric acid.

A number of dissections of pig, rabbit, and sheep embryos were made. Most of these were treated according to the method given by Prentiss ('15).

Opportunity to examine twelve human brains was obtained through the courtesy of Prof. S. W. Ranson. It was attempted to ascertain if the relations of the fibers of the nervus terminalis to the cerebral blood-vessels is the same in the human as in the cat, the mule, and the ox. The nerve was identified in five of the brains, but no definite light was obtained on the point in question.

ABBREVIATIONS

ant.cer.art., anterior cerebral artery
art.w., arterial wall
ax., axone
ax.cyl., axis cylinder
bi.c., bipolar cell
bl.v., blood-vessel
bu.olf., bulbus olfactorius
bu.olf.ac., accessory olfactory bulb
cen., centripetally
cer.hem., cerebral hemisphere
co.ant., anterior commissure
c.pr., central process
cri.pl., cribriform plate
d.n.ter., dorsal main bundle of nervus terminalis in nasal septum
fi., nerve fiber
gn., main ganglion ('ganglion terminale') of nervus terminalis
gn'., accessory ganglia
in.pl., intracranial plexus of nervus terminalis
lam.ter., lamina terminalis
m.n.ter., median main bundle of nervus terminalis in nasal septum
my., myelinated nerve fiber
my.sh., myelin sheath
n.eth.ant., anterior ethmoidal nerve
n.op., optic nerve
no.R., node of Ranvier
n.ter., nervus terminalis
n.vom., nervus vomeronasalis
olf.fi., olfactory fibers
op.chi., optic chiasma
or.vom., vomeronasal organ
per., peripherally
p.pl., peripheral (septal) plexus of nervus terminalis
p.pr., peripheral process
pr., nerve process
R., fiber of Remak
r^1,r^2,r^3,r^4, central roots of nervus terminalis
ram., ramus
r.dor., ramus of dorsal main bundle of nervus terminalis
resp.epith., respiratory epithelium
r.med., ramus of middle main bundle of nervus terminalis
r.ven., ramus of ventral main bundle of nervus terminalis
sp., spiral
un.c., unipolar cell
unmy., unmyelinated nerve fiber
v.n.ter., ventral main bundle of nervus terminalis

Turtle embryos and young of Amia were prepared by various methods and a number of dissections were also made.

For nerve terminations the gold-chloride method was employed, according to the modification given by Hardesty. Methylene-blue was tried, but with less success in demonstrating the sensory terminations to be described, although it brought out

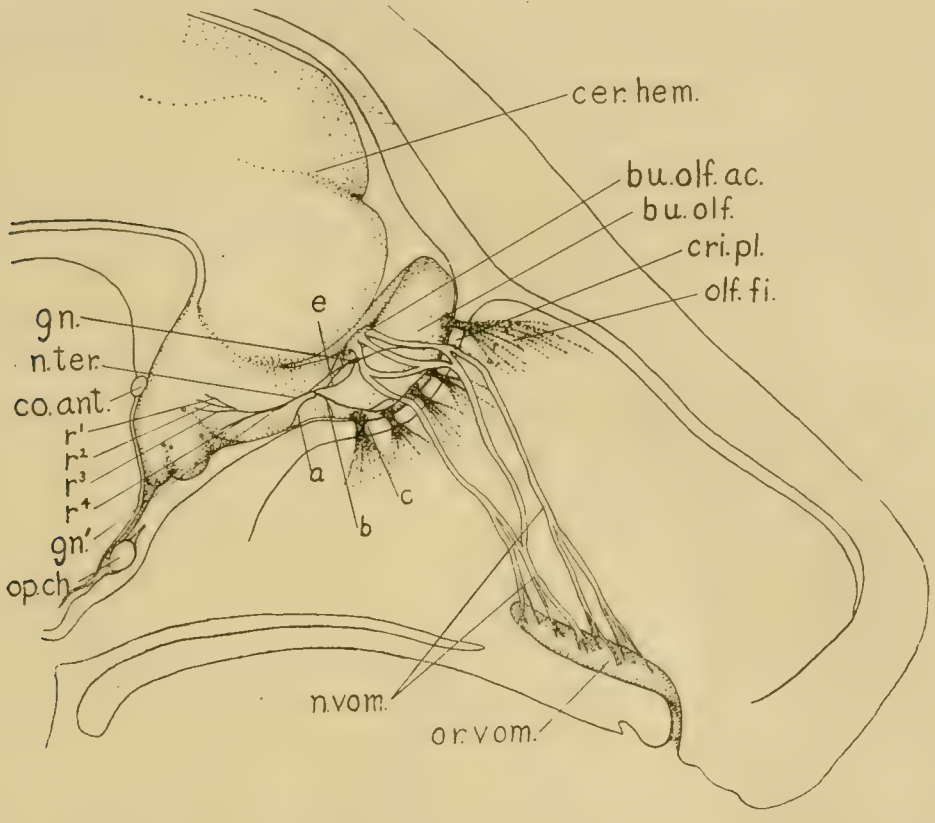


Fig 1 Dissection of nervus terminalis of kitten of two weeks, illustrating plexiform appearance of nerve, as seen through the binocular microscope. The blood-vessels, along which the majority of the nerve strands course, are not represented.

the motor endings quite clearly. Both sensory and motor terminations were shown in pyridin-silver preparations.

A modification of the pyridin-silver method given by Huber and Guild ('13) was used with good results. This modification consisted essentially in lengthening the periods during which the preparation was kept in the various fluids. Briefly summarized, the procedure was as follows: Ammoniated absolute alcohol

(after injection with same) seven days; decalcification in 7 per cent nitric acid; washing; 80 per cent, 95 per cent, and absolute alcohols with 1 per cent ammonia added to each, ten days altogether, to insure thorough dehydration; pyridin four days; silver nitrate, after washing, ten days; four per cent pyrogallol in 5 per cent formalin two days. All of these fluids except the last, were changed several times. In several of the preparations the strength of the silver solution was varied, beginning with a solution of 2 per cent for several days, then reducing to 1 per cent, to 0.75 per cent, and finally back to 2 per cent. The results in the way of details of structure and of staining the finer fibers, which were obtained by this modification were superior to those given by the unmodified procedure.

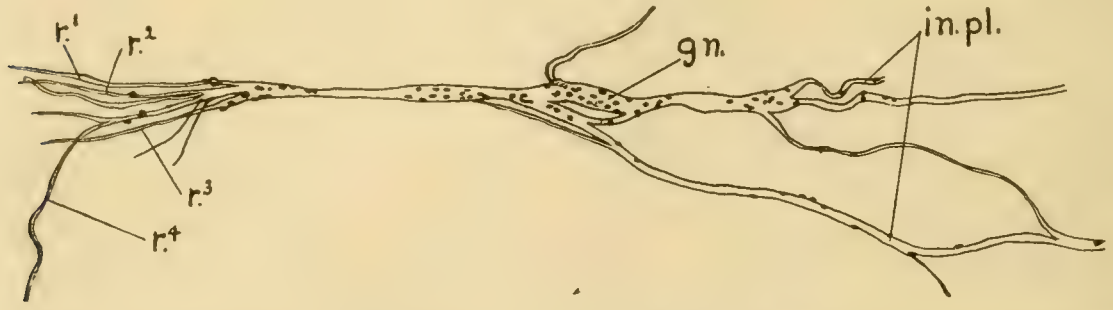


Fig. 2 Right nervus terminalis from same kitten of which the left nerve is shown in figure 1. Removed and mounted entire. × 32.

1. The nervus terminalis of the cat

The nervus terminalis of the cat is found on the medial side of the olfactory stalk. The main trunk runs parallel with the ventral border of this stalk, between the fissura prima of the forebrain and the vomeronasal nerves. As shown in figure 1, which represents the mesial aspect of the forebrain and nasal septum of a kitten of two weeks, the nerve is connected with the brain by three strands (r^1,r^2,r^3). They follow closely parallel to blood-vessels of small calibre, which enter the brain near the fissura prima. When these vessels were cut at their points of entrance into the brain, the nerve strands also became detached. This was due to the minuteness of the strands which it was not found possible to sufficiently disentangle from the connective tis-

sues surrounding both vessels and nerves. Sections (figs. 3 and 23) indicate that the larger strands at least enter the brain independently of the blood-vessels.

A fourth strand (r^4), which joins the nerve trunk, appears not to enter the brain. This strand was traced caudally for a short distance along the anterior cerebral artery, but became so attenuated by separating into minute bundles of fibers that it was not possible to follow the divisions far.

In one of the specimens examined, a kitten one day old, cells similar to ganglion cells were observed along the course of the roots for a little distance within the brain (fig. 23).

An elongated ganglionic swelling (fig. 1, *gn'*) is shown rostrad to the point where the nerve strand from the anterior cerebral artery unites with the main trunk of the nerve. Further rostrad a larger ganglion (*gn*) is seen in close proximity to the most caudal of the three principal vomero-nasal bundles.

Between these two ganglionic masses the main trunk of the nerve breaks up into a plexus of nerve strands (*a*, *b*, *c*). Many of these follow the larger blood-vessels of the region and send twigs into their walls. Three of the largest strands of the plexus converge distally, uniting with vomeronasal bundles. A number of strands, finer than any represented in the figure were found, but were torn in dissection. They were composed of relatively few fibers each, and uniting with the larger strands, formed a loose plexus over the medial surface of the olfactory bulb, as shown in figure 3.

The more ventrally located (*c*) of the larger bundles divides into two strands which unite, one with the ventral bundle of the vomeronasal nerve, the other with a more dorsally located bundle of the same nerve.

While the three strands (*a*, *b*, *c*), already noted, diverge at various angles from the principal axis of the nerve, the other divisions do not depart so widely. As shown in figure 1 (e), they form a secondary plexus which reunites, with the exception of one small strand, into the ganglionic swelling (*gn*) already noted. The single bundle which continues rostrally from this ganglion crosses two of the vomeronasal bundles to become en-

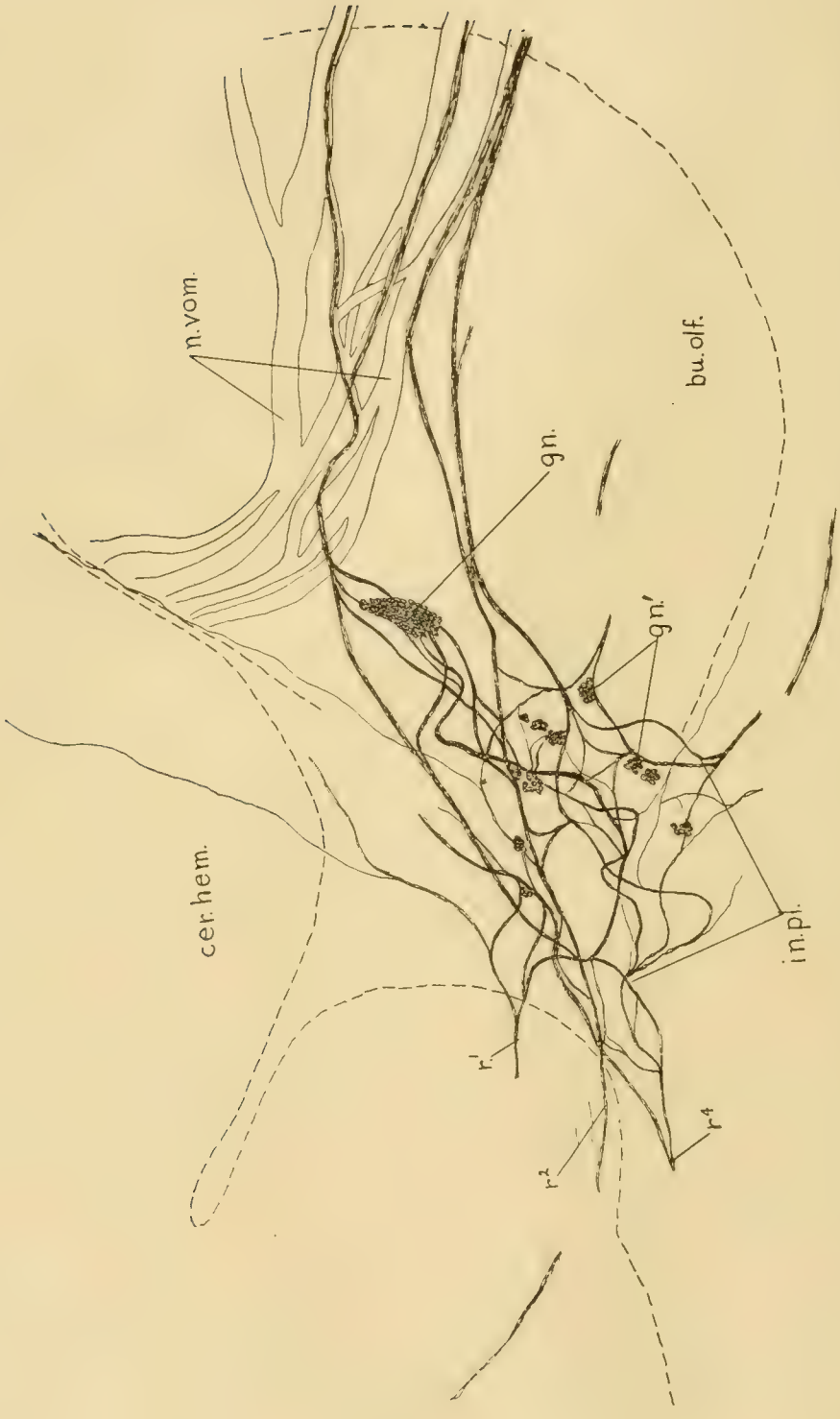


Fig. 3 Reconstruction of the intracranial plexus of the nervus terminalis projected on one plane from a series of sagittal sections through forward part of the head of two-weeks-old kitten. Pyridin-silver technique.

cased within the same connective tissue sheath which surrounds the third, more rostrally situated, vomeronasal bundle. The small strand which fails to reunite with the plexus appeared to have been torn from its course distally along a blood-vessel which passes between the dorsal surface of the olfactory bulb and the cerebral hemisphere.

Essentially the same relations to the vomeronasal nerves and to the cribriform plate were found in a dissection of a half-grown kitten, not figured. In this specimen the left olfactory bulb was removed, and it was attempted to trace strands of the terminalis into the nasal septum. Six strands which clearly belong to the nervus terminalis were present. Four of these became related to the vomeronasal nerves, and one of these four remained sufficiently separated from the dorsal bundle of this nerve so that its course could be followed distinctly through the cribriform plate. Most of the strands became enclosed by the sheaths of the vomeronasal bundles in such a manner that it was not possible to distinguish them from the strands of the latter nerve in their course peripherally by the method of dissection. Two of the strands which passed more dorsally did not converge with the vomeronasal bundles. One of these passed through one of the more dorsally situated foramina of the cribriform plate, together with a large olfactory bundle, and its course on the nasal septum was traced for some distance. The other continued dorsally and became attached to an artery which lay in the furrow between the olfactory bulb and the cerebral hemisphere.

Figure 3, which represents a graphic reconstruction of the terminalis plexus between the vomeronasal nerves and the point where its roots enter the brain, shows essentially the same relations. This figure represents a composite of thirteen sections of the region of the forebrain and nasal septum of a kitten two weeks old, prepared by the pyridin-silver method. It supplements figure 1 by showing the finer strands of the plexus to which reference was made, and by bringing out numerous small ganglia which could not be seen in the dissection represented in figure 1. A comparison of figure 3 with figure 2, which represents an in toto amount of the right nervus terminalis of the same

kitten from which figure 1 was drawn, is interesting in showing the same manner of distribution of the larger strands as is seen in the reconstruction. It also indicates, somewhat more clearly, the position of the ganglion cells along the main trunk of the nerve. This specimen differs from the one illustrated in figure 3 in that the main ganglionic mass (*gn*) has fewer ganglion cells than are present in the corresponding ganglion (*gn*, fig. 3) of the other kitten of the same age. More numerous cells, however, are scattered along the nerve trunk, so that the total number is approximately the same in the two specimens, if the smaller ganglia, not observed in the dissected animal, are left out of consideration in both.

The finer strands, which radiate in various directions from what may be designated the central bundle, follow along or soon reach, blood-vessels of various sizes. Many similar strands, consisting of but three or four fibers could not be seen with the low magnification of the projection apparatus used in plotting the figure, and are not included in this reconstruction. These, if represented, would make the plexus much more intricate, especially in its rostral part, and would cause it to extend further rostrally over the olfactory bulb than is figured.

The course of the nervus terminalis in the nasal septum was followed to best advantage in methylene-blue preparations, fixed in ammonium-picrate and mounted in a mixture of ammonium picrate and glycerine. The silver preparations brought out more clearly the finer strands, but the distortion of the septum produced by this technique made it difficult to follow the general course of the various branches by the method of reconstruction.

The nasal septum of a kitten one day old was removed and was kept moistened for forty minutes in a 0.25 per cent solution of methylene-blue in physiological salt solution. The preparation was examined from time to time until a differentiation was observed between the main bundles of the vomeronasal nerves and the smaller bundles which course parallel to them and which had previously appeared to be part of them. These smaller bundles assumed the blue color characteristic of this stain, while the vomeronasal nerves remained practically unstained. After

fixation over night in ammonium-picrate, the mucosa was removed from the bony septum and was mounted whole. This was done with the mucosa from both sides of the septum. The two sides showed essentially the same picture, but the right side was somewhat clearer, and is illustrated in figure 4.

In this specimen the vomeronasal nerves (*n.vom.*) consist of three principal bundles in their proximal course on the septum. About midway toward the organ of Jacobson, which could not be removed with the mucosa without too great danger of injury to the latter, two of these bundles divide into secondary strands. These strands continue to the vomeronasal organ. Parallel with the vomeronasal bundles and in close proximity to them for some distance are the main strands of the nervus terminalis. These strands pass through the cribriform plate, as previously shown in the dissections and as verified by pyridin-silver preparations, in company with the vomeronasal bundles. They continue parallel with them for some distance (fig. 4, *n.ter.*) and then divide, forming an intricate plexus in the deeper part of the mucosa (fig. 4, *p.pl.*). Comparison with silver preparations of the nasal septum makes it evident that only a portion of the plexus was stained in this specimen. This portion was derived chiefly from the most dorsal (*d.n.ter.*) of the principal terminalis strands present. The median of these strands (*m.n.ter.*) gives off some small twigs of fibers which anastomose with the main trunk of the dorsal bundle, and more rostrad it breaks up into branches, one of which (*r.med.*) forms a portion of the plexus. A larger branch of this median bundle continues parallel with the median branch of the vomeronasal bundle, but could be followed for only a short distance rostrally. The most ventral bundle of the terminalis (*v.n.ter.*), which is also the largest, was lost distally because of the idiosyncrasy of the stain. A small twig (*r. ven.*) given off in the more proximal part of its course passes beneath the ventral vomeronasal bundle and is soon lost in the mucosa ventral to this bundle. A large branch (*r.dor.*) from the dorsal terminalis bundle also courses ventrally, but this could not be traced beyond the dorsal ramus of the ventral vomeronasal bundle. All other branches which were stained

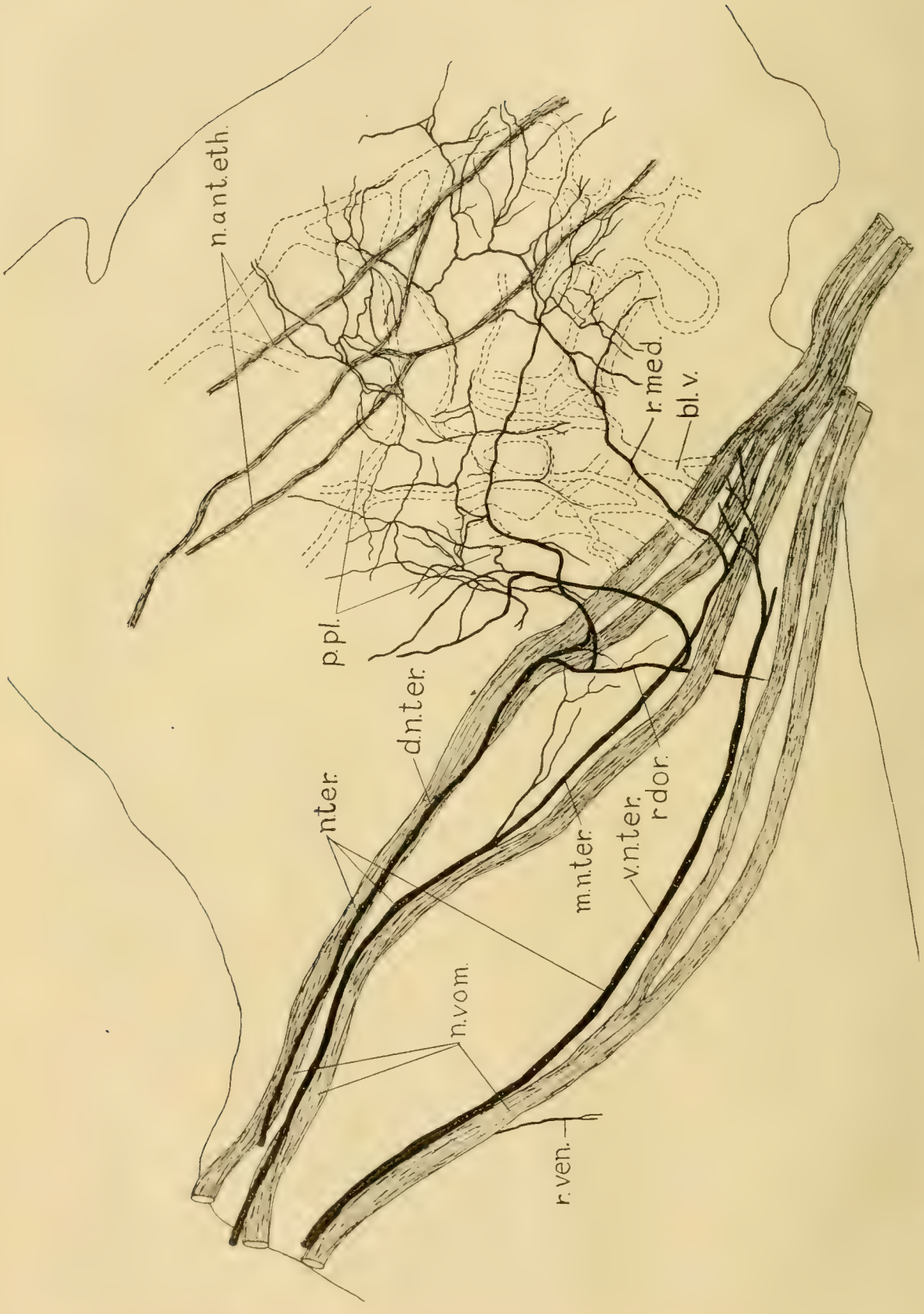


Fig. 4 Peripheral distribution of nervus terminalis on nasal septum of a kitten one day old. Methylene-blue stain. Whole mount. × 20.

coursed toward that part of the mucosa which lay dorsal to the vomeronasal bundles, where the greater part of the plexus is located. Examination of the figure indicates that many of the nerve strands follow quite closely the paths of the blood-vessels represented by broken lines. Owing to the complexity of the vascular network, only the larger of these vessels were seen clearly enough in the preparations to make it possible to trace their courses. No ganglion cells or small ganglia were seen, but this was laid to the peculiarity of the stain. Silver and Weigert preparations revealed the presence of such cells in the nasal septum, but in much smaller numbers than are indicated in the rabbit by Huber and Guild ('13) or in the human by Brookover ('17). The ganglion clusters are, however, very numerous intracranially, especially on the mesial sides of the olfactory bulb.

While it seems likely that most of the plexus formed by the nervus terminalis on the nasal septum was seen, it is doubtless true that the rostral part of the septum, which unfortunately did not take the stain, also contains a continuation of this plexus. Pyridin-silver preparations indicate this beyond question in the cat, and it has been shown to be true in the rabbit by Huber and Guild, and in the human by Brookover, in the papers above cited.

The observations of the olfactory region of the mucosa are more dubious. In the methylene-blue preparations, the region in which olfactory fibers were present was stained a diffuse dark blue-green, which made it impossible to see any portion of the plexus if it were present. The pyridin-silver material shows occasional fibers in this region which may belong to the nervus terminalis, but this cannot be stated with any degree of certainty. It is possible that they are fibers from the anterior ethmoidal nerve, the main trunk of which lies in close proximity to many of the fibers found.

So far as the methylene-blue material indicates, there is no connection of the nasal plexus of the terminalis with either the anterior ethmoidal or the nasopalatine branches of the trigeminal nerve. The silver preparations, however, showed such a confusion of trigeminal and terminalis fibers in the rostral end

of the septum that it seems certain that there is some commingling of fibers from the nasopalatine nerve in the terminalis plexus. This was also indicated, although somewhat less clearly, in Weigert preparations. The Weigert material showed very clearly that fibers from the anterior ethmoidal nerve take some

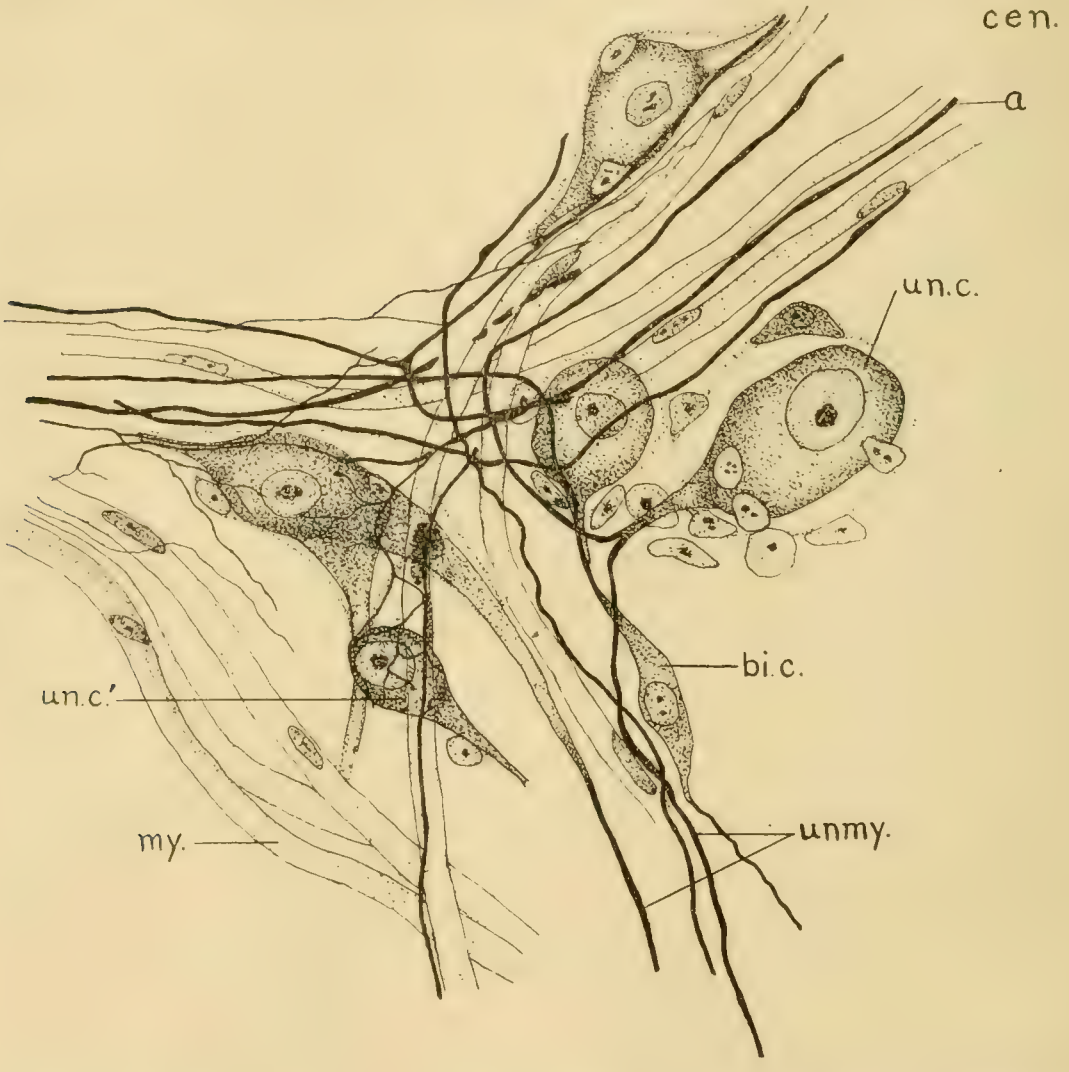


Fig. 5 A small cluster of cells slightly posterior and ventral to the ganglion (*gn.*) of figure 3, illustrating some of the types of ganglion cells, together with myelinated and unmyelinated fibers. Pyridin-silver technique. × 825.

part in the formation of the peripheral plexus of the nervus terminalis. As shown in figure 22, which represents a portion of this plexus in a kitten one day old, a few myelinated fibers are present. Some of these were traced into a strand of the anterior ethmoidal nerve, which lay in close proximity to the

portion of the plexus figured. This nerve shows development of the myelin sheaths, while the intracranial portion of the nervus terminalis of this specimen (fig. 23) gave no indication of myelin sheaths as brought out by the Weigert treatment. It does not seem likely that such sheaths would be formed in the peripheral portion of the nerve at an earlier date than they are formed in the part of the nerve nearer the brain. It is therefore assumed that they are fibers belonging to the already myelinated anterior ethmoidal nerve.

So far as this material indicates, the central roots of the terminalis, which are easily followed in the sections to their points

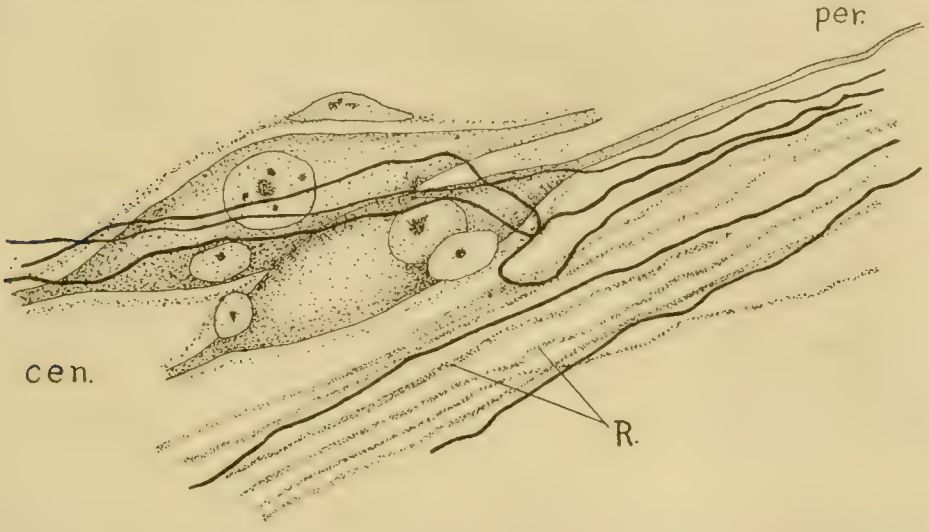


Fig. 6 Two bipolar cells and some of the nerve fibers from periphery of main ganglion (fig. 3, *gn.*) of the nervus terminalis in kitten of two weeks. Pyridin-silver technique. × 1266.

of entrance into the brain, are composed entirely of unmyelinated fibers. In kittens of two weeks, myelinated fibers are found in the intracranial plexus, although in these also no clear evidence of such fibers was found among the strands which enter the brain.

To avoid as far as possible the entrance of fibers from the fifth nerve as a factor, the greater part of the histological studies to be described was confined to the intracranial plexus and ganglia of the terminalis.

Histological. The plexiform character of the nerve in the cat, together with the structure of the ganglion cells to be described

in the mule, and the relation of the nerve to the adjacent blood-vessels shown in cat, mule, and beef, suggested strongly that the nervus terminalis is composed, at least in part, of sympathetic fibers. There remains the possibility, which could not be adequately tested in the available equine or bovine material, that there may be a general or special sensory component, in addition to the motor and sensory sympathetic fibers which were found. It was accordingly deemed advisable to make as thorough a study of the terminalis ganglia and of the fibers connected with them in the cat as the material available would permit.

The fact that the nerve is situated in a position so difficult of access, together with its small size, and the further circumstance of its relation to the cribriform plate, made necessary a technique permitting of decalcification, so that nerve and ganglia might be studied in situ. For this reason, chiefly, the pyridin-silver method, as previously described, was employed.

There was considerable variation in different parts of the same section in the intensity and clearness of the impregnation. It was also found that, in general, the cells of the smaller ganglionic clusters were much better differentiated from the background than were those in the more crowded larger ganglia. Because of this fact the majority of the cells figured are from the small clusters of cells, but for comparison, considerable attention was paid to the large ganglionic mass (figs. 1, 2 and 3, *gn.*) which appears to correspond with the 'ganglion terminale' of authors.

Types of ganglion cells. Figure 5 represents a typical small ganglionic mass which had its position in the meninges covering the mesial side of the olfactory bulb. It lay slightly caudad to the ganglion terminale and a little more ventrally. This cluster was similar to numerous others scattered throughout the plexus. Such clusters of cells are usually situated at the meeting point of several small strands of fibers which converge from various directions. The group of cells figured represents only a portion of this particular ganglionic mass. The remaining portion was to be seen in the next section of the series.

It will be noted that both myelinated (*my.*) and unmyelinated (*unmy.*) fibers are present. The axis cylinders of the myelinated fibers were stained a darker orange color than were the myelin sheaths surrounding them. The processes of the unmyelinated fibers were quite black and stood out distinctly. One of the latter (*a*), which comes from the direction of the central connection of the nerve, divides into two smaller fibers which in turn subdivide into terminations with small varicosities, and which form simple pericellular baskets on two of the nerve cells shown.

Several classifications, both of ganglion cells and of sympathetic cells, have been made by investigators, notably by Cajal ('05) Dogiel ('08), and Ranson ('12) for the former; and by Cajal ('05), Carpenter and Conel ('14), Dogiel ('96), Michailow ('11), and others for the latter type. There is considerable individual variation among the ganglion cells observed in the terminalis clusters, and they come within one or the other of these classifications. Still, for purposes of description it is convenient to designate the types observed according to the number of processes they possess as unipolar, bipolar, and multipolar. A few binucleated cells were seen in the cat, but aside from the number of nuclei, they resembled the other cells of the several varieties and will not be treated separately.

(*a*) *Unipolar cells.* Most of the unipolar cells observed resemble those usually considered characteristic of the spinal ganglia. The body of the cell (figs. 5, 10, 18) is ovoid or spherical, with a rather large nucleus. The single process, which usually stained brown near the cell body, becomes darker as it assumes a smaller diameter in its course away from the perikaryon. In those cases in which it was possible to follow it for any distance, it divides into two processes, one directed in the general direction of the central connection of the nerve, the other peripherally as respects this connection. No marked difference in size of these two divisions was noted, although that which appeared to be the central process was usually slightly smaller in diameter. It must be understood that only the general direction of the course of these fibers is indicated, because of the various directions different strands of the plexus assumed.

While myelinated fibers were present in the same bundles which included processes from the unipolar cells, in no case was a myelin sheath found in connection with processes from such cells.

Three distinct sizes of unipolar cells were observed. The predominating size is represented in figure 5 (*un.c.*), which also

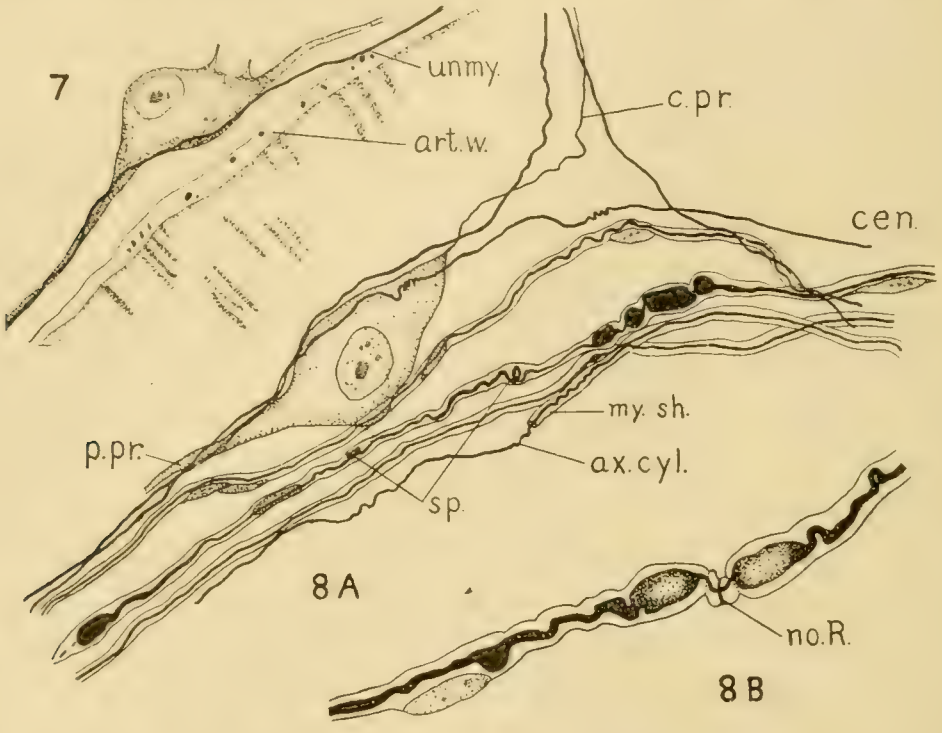


Fig. 7 Bipolar cell and accompanying unmyelinated fiber on the wall of a blood-vessel between the cerebral hemisphere and the olfactory bulb. Kitten two weeks old. Pyridin-silver technique. × 450.

Fig. 8A Characteristic small bundle of myelinated and unmyelinated fibers, with a single bipolar cell in its course. Fig. 8B Portion of a myelinated fiber more highly magnified, showing a node of Ranvier, and some large varicosities. Both from kitten of two weeks. Pyridin-silver technique. Figure 8A, × 660; figure 8B, × 950.

indicates very clearly the bifurcation of the single process of the cell. Figure 5 (*un.c'.*) also shows one of the smallest of the unipolar cells seen. The process of this cell could not be followed for any great distance and no bifurcation was seen. The process was directed peripherally. Attention may again be directed to the pericellular basket surrounding this cell. The relation of the cell to the nerve fiber of which this basket is the termination,

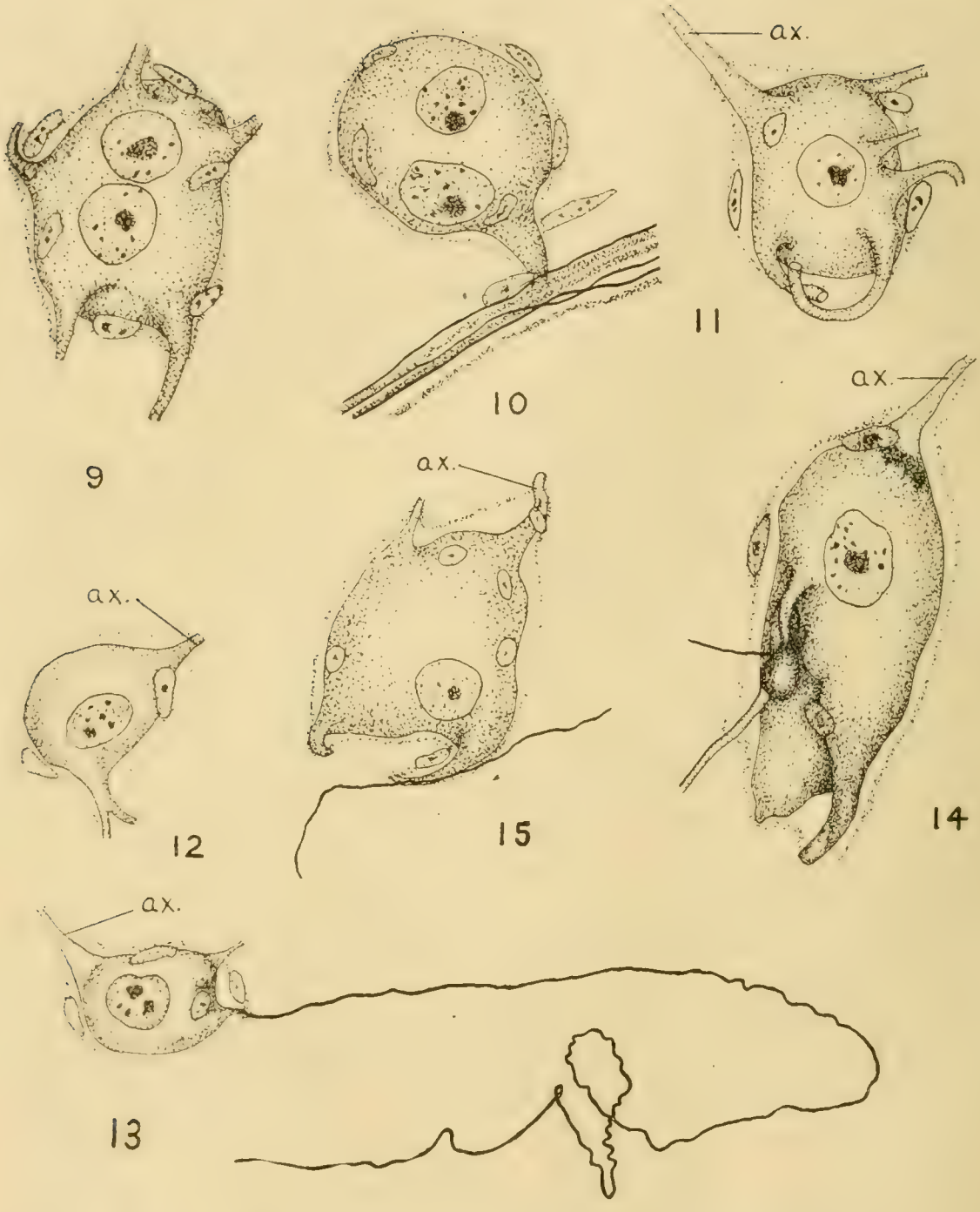
together with the peripherally directed process of the cell, suggests that it pertains to the sympathetic system. Its small size favors this interpretation.

The largest unipolar cell observed (fig. 10) was also the only one of this type found which was binucleated. The process was of large diameter and did not stain so dark as did the majority of fibers. It was not possible to follow it beyond its point of entrance into the bundle of smaller black fibers of which it became a part. The large size, both of cell and of cell process, resembles somewhat the large unipolar cells found by Carpenter ('12) in the ciliary ganglion of the sheep.

Unipolar cells were not numerous and were found only in the smaller ganglia. Whether their presence in the larger ganglia was hidden by the crowded condition of these could not be determined. It seems, however, that the latter are composed principally of multipolar cells, with fairly numerous bipolar cells near their peripheries.

(*b*) *Bipolar cells.* Cells of this type are quite numerous, both in the smaller ganglia and in the larger ones. A number of such cells isolated from other nerve cells were also found. Figure 6 represents two typical bipolar cells from the periphery of the largest ganglion (fig. 3, *gn.*) of the left nervus terminalis of a kitten of two weeks. The processes of these, which are of large size, took only a brownish tinge from the impregnation. A few fibers of small size which stained black were present in the immediate neighborhood of these cells and are indicated in the figure, as are also some large unmyelinated fibers. These are doubtless fibers of similar bipolar cells. A few relatively small bipolar cells were found, one of which is shown in figure 5 (*bi.c.*). The processes of these were quite slender and stained black. They were followed for some distance, but no conclusive evidence as to their terminations was obtained.

The isolated bipolar cells above noted were found in the course of small strands of fibers, between the nodal points where several such strands converge (fig. 8). A few were found on the walls of blood-vessels or near them. One of these is illustrated in figure 7, in which also is shown an unmyelinated fiber of small size

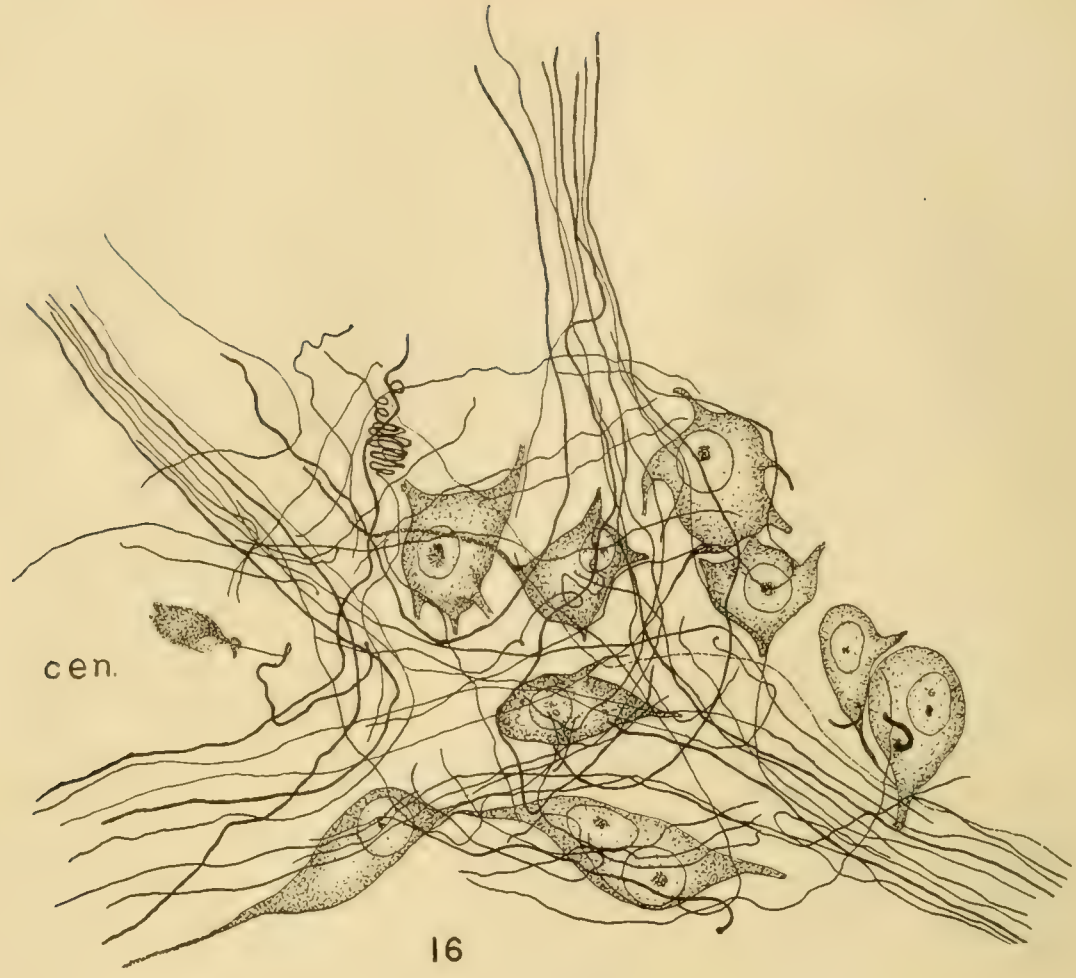


Figs. 9 to 15 Ganglion cells from intracranial clusters of nervus terminalis of kitten two weeks old. Figures 12 and 13 were drawn from cells which lay in the trunk of the nerve between its points of entrance into the brain wall and the main portion of the plexus; figures 14 and 15 were drawn from cells which lay near the center of the main ganglion (*gn.*) and the other figures were drawn from cells in various portions of the plexus. Figures 9, 10, 11, and 14 magnified 990 times; figures 12 and 13 magnified 1020 times, and figure 15 magnified 675 times. Pyridin-silver technique.

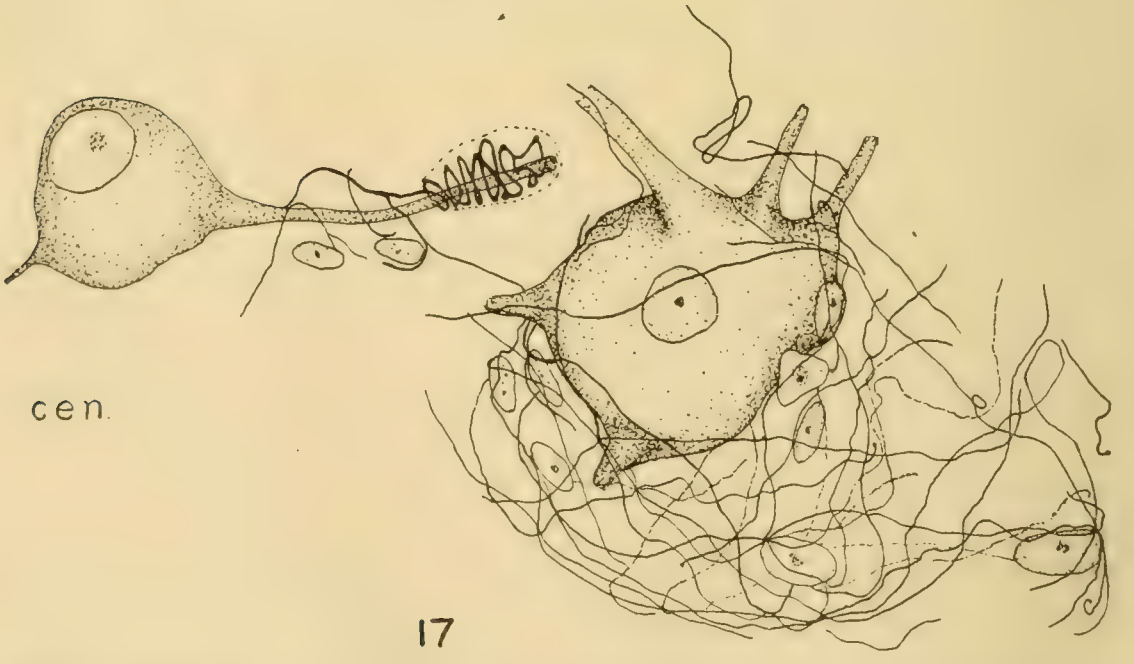
which runs parallel with the processes of the cell. The position of this cell was well within the crevice between the olfactory bulb and the cerebral hemisphere, on the wall of a blood-vessel which passes between the bulb and the hemisphere. The processes of the cell were stained rather lightly by the silver and it was not possible to follow them far. Their course so far as visible was parallel with the wall of the artery. Two slender processes may be seen to issue from the cell, in addition to the polar processes of much larger size. Because of these slender offshoots the cell should possibly be classed with those of multipolar type, but it is included with the bipolar cells because of its greater similarity in other respects.

In figure 8A is shown a cell whose position was on the medial surface of the olfactory bulb, posterior and ventral to the main ganglion of the terminalis. The larger process (*p.pr.*) is directed toward the ganglion, while the very slender process from the opposite pole of the cell is turned in the general direction of the central connection of the nerve with the brain, although the group of three fibers of which it forms one, turns at right angles to the principal axis of the terminalis plexus. This process was followed for some distance, but it was lost in the plexus centripetally.

(*c*) *Multipolar cells.* The multipolar type of cell predominates in the ganglia. These cells vary in size from the relatively small ones shown in figure 16, to the large cells drawn to the same scale represented in figures 9, 11 and 17. The number of processes varies. The cells illustrated in figures 12 and 13, which were found on the main trunk of the nerve, show but three processes. The majority of multipolar cells included in the small ganglia have at least five offshoots. Typical examples of such cells are shown in figures 11 and 16. The axone could not always be determined with certainty, but in the cells shown in figures 11, 12, 14, and 15 the process marked *ax.* appeared to be the axone. In each of these cases it was directed centripetally. That which appeared to be the axone of the cell shown in figure 13 (*ax.*) was directed peripherally. This cell lay in the course of the main trunk of the nerve.



16



17

Fig. 16 Small ganglionic cluster and intercellular network from portion of intracranial network of nervus terminalis near lower posterior border of olfactory bulb. Kitten two weeks old. Pyridin-silver technique. × 990.

Fig. 17 Large multipolar cell with extracapsular network from intracranial plexus of nervus terminalis of kitten two weeks old. Pyridin-silver technique. × 990.

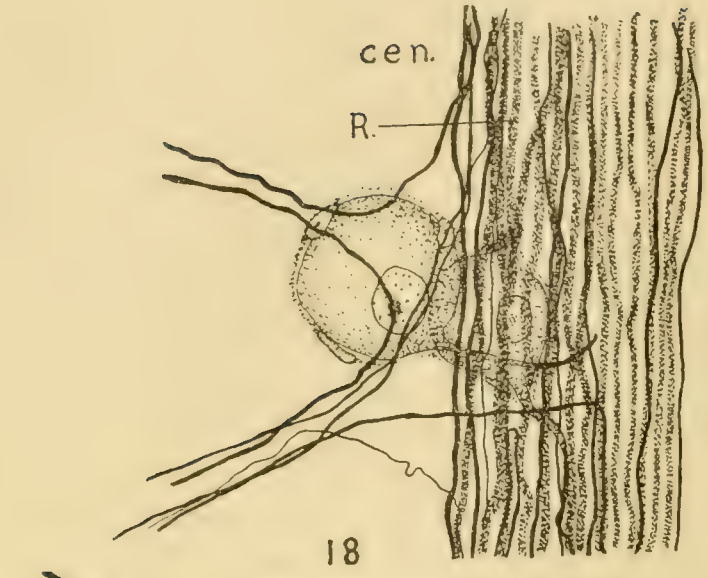
Very few binucleated cells of the multipolar type were observed. These, as illustrated by the one represented in figure 9, were similar in every other respect to other multipolar cells.

Fibers and fiber networks. A few of the individual cells were surrounded by a reticulum of delicate fibers which suggest an extracapsular network (fig. 17). The capsule itself was not easy to see in such cases, but the position of the capsule nuclei within the network seems to justify the interpretation given to these reticula. In the example illustrated, a small number of threads extend from the region of a neighboring bipolar cell and take part in the formation of the pericellular plexus described. The main fiber from which these threads have their origin, runs parallel to the larger process of the bipolar cell, and appears to terminate in a spiral (fig. 17) about this process. The fibers forming this network are intertwined in the most confusing manner in every direction. They are of very small size.

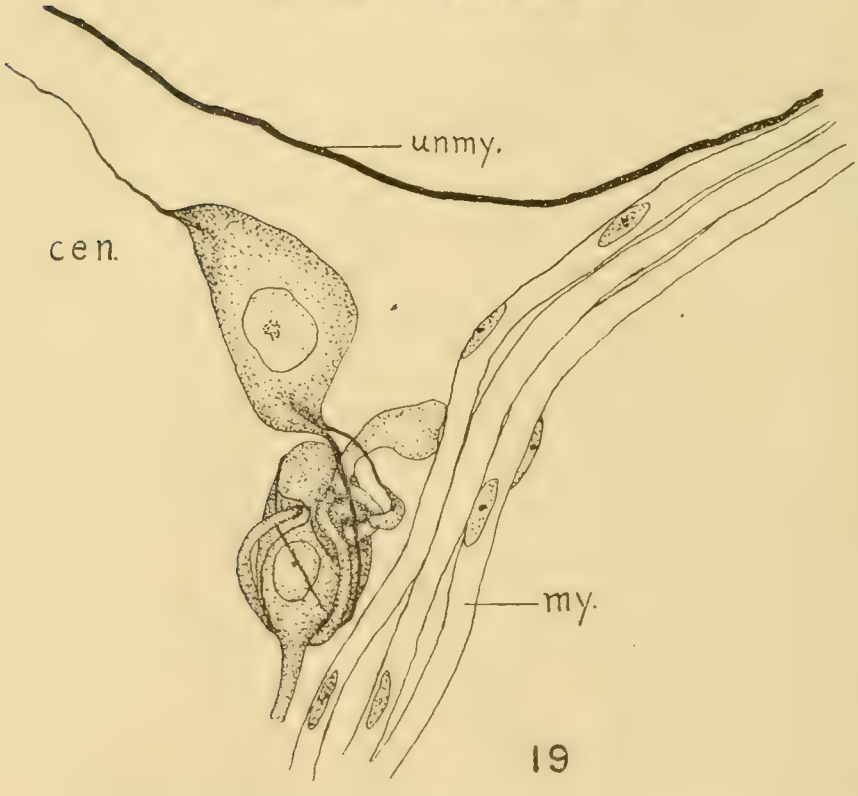
Intercellular networks also were found in those parts of the preparations where the impregnation was most favorable. A peculiarity of the impregnation revealed itself in the fact that those parts of the sections which showed the fibers most clearly did not serve so well to differentiate the outlines of the cell bodies and of the large processes from the perikaryon.

The intercellular networks were found in every case at nodal points where a number of fiber strands converge. Usually the ganglion cells enclosed by such a network were of small size, but occasionally a larger cell was also included. In the example illustrated (fig. 16), which was situated near the lower margin of the posterior portion of the olfactory bulb, four strands of fibers converge about a small group of cells. Many of the individual threads may be followed from one strand through the cell cluster and into one of the other converging strands, without any apparent connection with the cells. The majority of fibers were lost in the network. A few may be seen to connect with nerve cells of the cluster.

This intercellular network resembles to a considerable degree structures of a similar nature found by Dogiel ('95) in the digestive tract of the dog. It bears an even more striking resem-



18



19

Fig. 18 Bundle of fibers with two cells in its course, and showing the characteristic method by which small strands of fibers leave the larger bundles. Kitten two weeks old. Pyridin-silver technique. × 675.

Fig. 19 A somewhat isolated strand of myelinated fibers and a single unmyelinated thread of relatively large size as compared with the centrally directed process of the nerve cell shown. Kitten two weeks old. Pyridin-silver technique. × 990.

blance to networks found by Ranson and Billingsley ('18) in the cervical ganglion of the dog.

An attempt was made to analyze the various strands of the plexus, in order to determine, if possible, the types of component nerve fibers. As previously stated, both myelinated and unmyelinated fibers are present. Of the latter type, both varieties, namely, fibers of Remak and naked filaments, are abundant. While for short stretches some of the strands appear to be composed exclusively of one type or the other (figs. 5, 6, 8, 19), the rule is mixed strands. The fibers of Remak predominate as to number when the entire plexus is considered. They are particularly numerous near the main ganglion, and appear to include the majority of fibers which enter this ganglion.

In figure 20 are shown two converging strands consisting principally of Remak's fibers, which approach this ganglionic mass. The bundle resulting from their union was one of the most compact of the entire plexus. Naked filaments of several sizes are also included in it. One of the most slender of these (*fi*) is seen to approach the nerve cell represented in the figure and to follow what appeared to be the axone of the cell, to end on the perikaryon in a simple pericellular termination. A similar strand, but with fewer naked fibers, is shown in figure 21, which was drawn from near the ventral border of the posterior part of the olfactory bulb. This bundle of fibers runs parallel with one of the arteries of this region. An offshoot (*ram.*) consisting of four or five threads leaves the main strand and passes to the wall of a branch of the artery.

Two fibers which show no neurilemma sheath leave the strand to pass to the wall of the main artery. These strands so closely resemble others, the terminations of which are described below, that it seems certain that they represent fibers which ramify to form the type of nerve-endings shown in figures 24 to 29. Whether or not the fibers of Remak terminate in the sensory endings represented in figures 28 and 29 could not be determined with certainty. It seems unlikely, in view of the fact that the fibers leading to the sensory terminations show myelin sheaths near their endings. The naked filaments show considerable

variation in size, the finer fibers greatly outnumbering those of coarser diameter.

The myelinated fibers are relatively few in number. They usually occur in strands of three to five filaments, accompanied

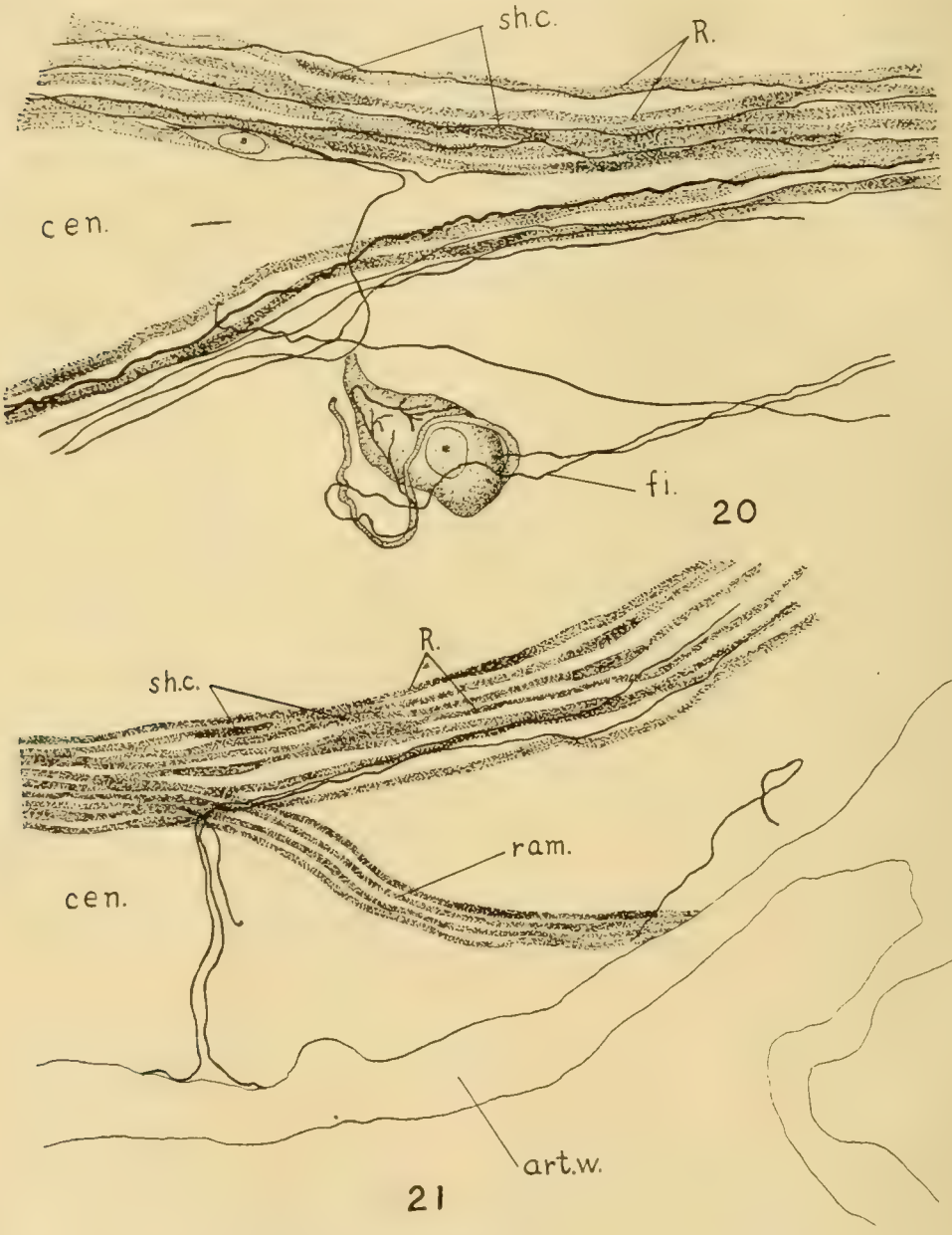


Fig. 20 A bundle of fibers from near the dorsal margin of the olfactory bulb, caudad to the main ganglionic mass. The strand divides centrally. Kitten two weeks old. Pyridin-silver technique. × 600.

Fig. 21 A bundle of fibers from near ventral margin of olfactory bulb in about the same vertical plane as figure 20, showing relation of some of the fibers to a blood-vessel. Kitten two weeks old, Pyridin-silver technique. × 600.

by naked threads. In some cases these naked filaments represent fibers which have lost their myelin sheaths (fig. 8A, *ax. cyl.*). Many of them show very large varicosities (fig. 8, A and B), the most pronounced of which are often found near the nodes of Ranvier. These varicosities appear to have been produced by unequal shrinkage of the axis cylinder, probably during the process of fixation of the tissue. At other points, as shown in the figures, the cylinders are extremely attenuated. Some show spiral formations of the axis cylinder within the myelin sheath (fig. 8A, *sp.*), In diameter the intracranial myelinated fibers varied from 1.5 μ to 2.6 μ.

In the septal plexus of the terminalis, myelinated fibers are found intermingled with the unmyelinated threads (fig. 22). As previously stated, these belong, in part at least, to the trigeminus. It is possible that the myelinated fibers of the intracranial plexus are related to those found in the septal plexus, and may therefore be trigeminal in origin. This does not seem likely, but can only be adequately tested by degeneration experiments, which the writer hopes to perform.

The roots which enter the brain, in both of the extra-uterine stages of growth of the cat in which this point was examined, appear to be composed exclusively of unmyelinated threads (fig. 23). Fibers of Remak predominate, but a few naked filaments are mingled with them.

Nerve terminations. There are present in the cat two kinds of nerve terminations in the walls of the cerebral blood-vessels, which are connected with fibers from the nervus terminalis. A third type consisting of free endings in the epithelium of the nasal septum appears also to be related to this nerve.

The nerve terminations in the walls of the anterior cerebral artery and its branches, for convenience of description, will be designated as type I and type II.

Type I (figs. 24 to 27) consists of delicate varicosed fibers which penetrate the muscular walls of the blood-vessels from the nervous plexus surrounding these vessels. At varying depths in the muscular layer, the fibers which penetrate the arterial coat ramify into very fine arborizations which pass between the smooth

muscle cells and end on the latter. The nerve fibers which end in this manner are in every case unmyelinated. They show very slight typical varicosities. The twigs of the terminations are

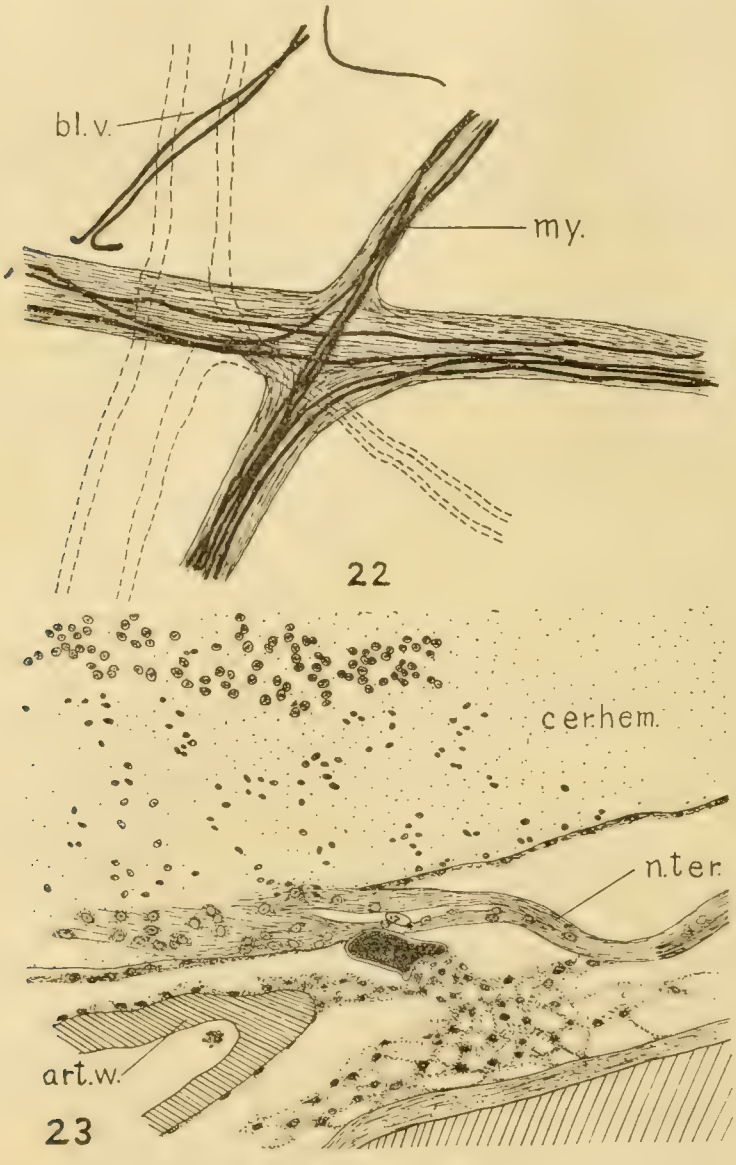


Fig. 22 Portion of septal plexus showing the presence of myelinated fibers. Midway between principal vomeronasal foramen and rostral end of nasal septum. Kitten one day old. Weigert technique. × 230.

Fig. 23 Central roots of nervus terminalis at point of entrance into brain wall. Kitten one day old. Weigert technique. × 75.

also varicosed. It will be noted from the figures that the manner of distribution of these terminations varies considerably. Those represented in figures 25 and 27 end in relatively short,

stout twigs. Others (figs. 24 and 26) have long, very delicate branches, which sometimes continue their course parallel with the plane of the fibers from which they spring, sometimes di-

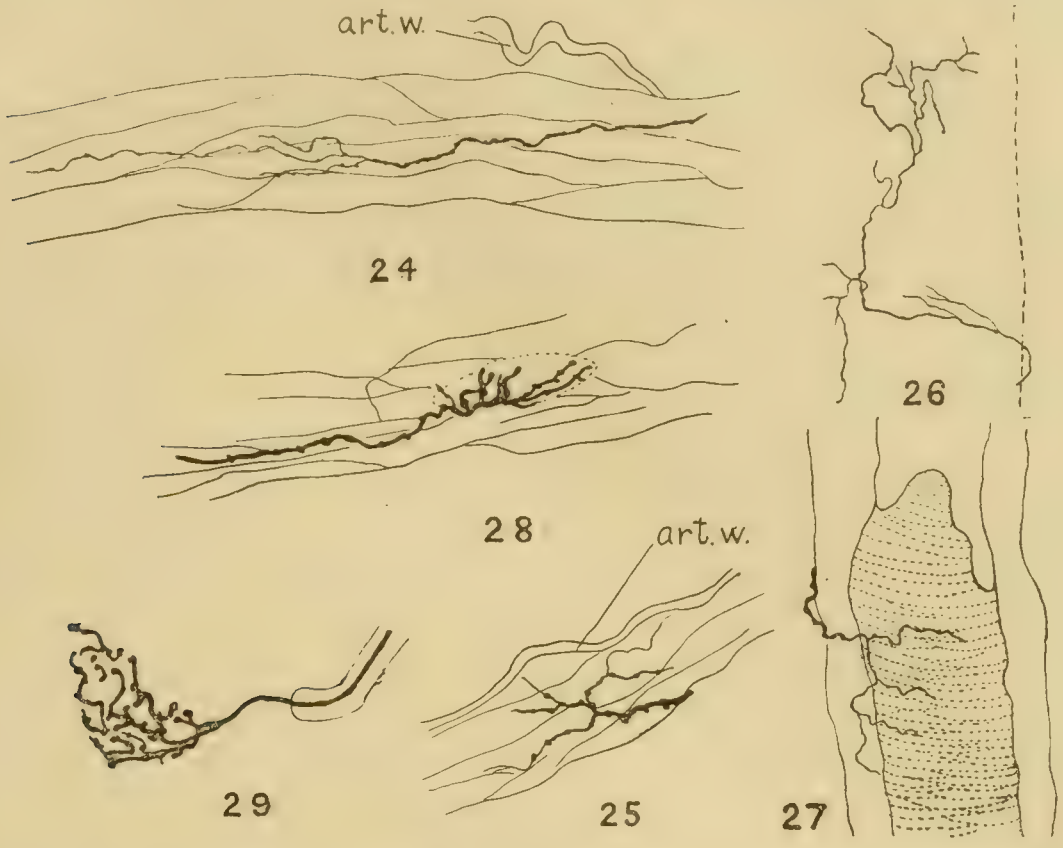


Fig. 24. Motor nerve termination from muscular coat of anterior cerebral artery of kitten two weeks old. Gold-chloride technique. × 1020.

Fig. 25 Motor nerve termination from branch of anterior cerebral artery of kitten two weeks old. Gold-chloride technique. × 1020.

Fig. 26 Motor nerve termination from one of the vessels on the mesial surface of the olfactory bulb (in pia mater) of kitten two weeks old. Pyridin-silver technique. × 1425.

Fig. 27 Motor nerve termination from one of the blood-vessels of the nasal septum of kitten one day old. Pyridin-silver technique. × 1425.

Fig. 28 Sensory nerve termination from anterior cerebral artery of kitten two weeks old. Gold-chloride method. × 1020.

Fig. 29 Sensory nerve termination from a small vessel in the meninges near the olfactory bulb of kitten two weeks old. Pyridin-silver method. × 1425.

verge from it at various angles. These terminations appear to be similar to those found by Huber ('99) in the cat, and considered by him to be motor endings.

The terminations represented in figures 24 and 25 were stained by the gold-chloride method. Those shown in figures 26 and 27 are from pyridin-silver preparations. Similar endings were also found by the molybdenum methylene-blue process, in the anterior cerebral artery and its branches, from which vessels all of the preparations were made.

The type II endings are strikingly different in appearance from those of type I. As shown in figures 28 and 29, the terminations are by somewhat spindle-shaped structures, composed of short, thick branches from the main fiber. These rami end with terminal knobs. In the gold-chloride preparations (fig. 28) a spindle-shaped clear space appears to be enclosed by the short processes which are derived from the nerve fiber. In the silver material no such clear space is evident, although the general contour of the termination is the same as in the gold preparations. The pyridin-silver slides showed the presence of delicate myelin sheaths on the fibers leading to these terminations. Such sheaths are not clearly evident in the gold chloride material of the cat, although similarly prepared slides of the corresponding blood-vessels of the beef indicate their presence in that animal. No capsules are present around these terminations in any of the animals in which they were examined. The methylene-blue staining did not clearly demonstrate this type of endings, although suggestions of them were visible by this method also.

In general appearance these end-organs resemble to some extent the corpuscles of Ruffini, but are much smaller. Both in shape, however, and in the absence of a capsule, they bear a stronger similarity to a type of sensory ending found by Dogiel in the heart of the cat (Dogiel, '96, fig. 2, *D*). Smirnow describes terminations of somewhat similar appearance in the atrial endocardium of the cat (Smirnow, '95, fig. 6), and Michailow ('08) has also described non-capsulated sensory terminations in the myocardium.

In the anterior cerebral artery and its branches of the cat and of the beef, they lie not in the loose connective tissue, but scattered at various levels in the muscular coat itself.

So far as the writer is aware, similar structures have not heretofore been described in the walls of the cerebral blood-vessels. Huber ('99) noted myelinated fibers along the walls of the cerebral arteries of the cat, and considered them to be sen-

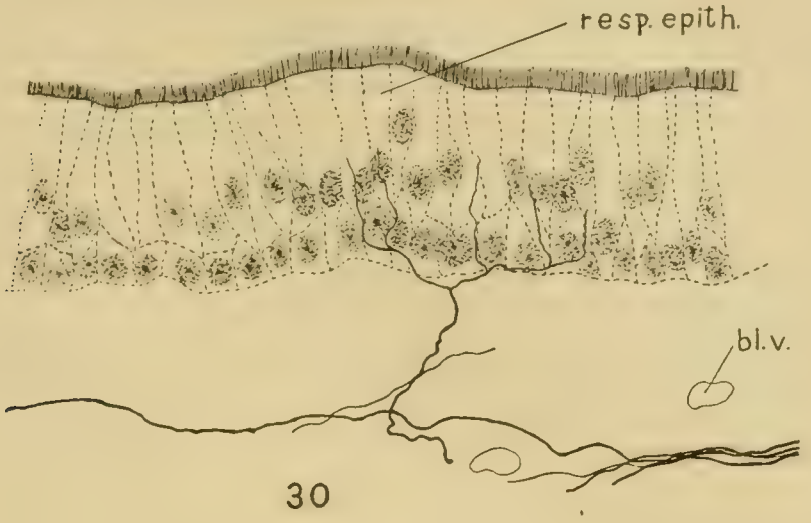




Fig. 30 Free nerve terminations in respiratory portion of nasal mucosa of kitten one day old, also a portion of the nervous plexus. Pyridin-silver technique. × 600.

Fig. 31 Portion of the septal plexus of the nervus terminalis of kitten one day old, showing one of the fibers bifurcated and ramifying into slender twigs which appear to have been cut off at their ends. Pyridin-silver technique. × 600.

sory as distinguished from the unmyelinated motor fibers which he found in company with them.

The compact form of this type of endings, differing to so marked a degree from the other type which has been described as motor, and closely resembling sensory terminations in other

parts of the vascular system, seems to justify the assumption that they are sensory in function.

The third type of ending to which reference was made was found among the epithelial cells lining the mucosa of the nasal septum. These endings consist of very delicate arborizations which pass between the columnar cells of the epithelium and approach the surface of the membrane (fig. 30). They are terminal twigs of fibers which appear to be unmyelinated. These fibers, as shown in the figure, approach the epithelium in small strands of three or four fibers to spread out at its base, where the terminal threads which form the free end fibers are given off. No varicosities or end-knobs were seen.

Such terminations are present in both sensory and respiratory regions of the septal mucosa and in the epithelium of the vomeronasal organ. Similar endings, but with varicosities or end-knobs, have been described and figured in these regions by von Brunn ('92), von Lenhossék ('92), Retzius ('92), Cajal ('94), Read ('08), and others. Most of these writers tend to ascribe them to the trigeminal nerve, although von Lenhossék suggests the possibility that they represent olfactory fibers whose cells of origin do not have the same position as others, but lie within the centripetal olfactory tract, enclosed in the course of the olfactory bundle.

Figure 31 represents what appears to be the centripetal continuation of a fiber which gives rise to free endings such as those just described. This figure was drawn from a section which lay just below the epithelium, in a sagittal series through the nasal septum. As shown in the figure, the fiber (*fi*) divides at its extremity into four slender twigs which appeared as if they had been cut near their tips. Centripetally this fiber unites with a similar one (*fi'*). The nerve process of which these fibers are branches is part of a small bundle (*p.pl.*) which forms a portion of the terminalis plexus of the nasal septum shown in figure 4.

While the fibers which terminate in the manner indicated resemble in size and distribution those of the nervus terminalis, there remains the possibility that they are the continuation of the more delicate threads which are present in the nasopalatine

and anterior ethmoidal branches of the trigeminal nerve. As already noted, there is a commingling of fibers of this nerve and of the terminalis, and fibers of the trigeminus enter the bundles which constitute the septal plexus of the terminalis. The intricacy of this plexus made it impossible to follow any individual fiber very far. Accordingly it was not possible to determine with certainty whether the free terminations of the septal mucosa are from terminalis fibers or from the trigeminal nerve.

Other fibers of larger size are also present in the mucosa. These terminate on or near the septal glands, and show varicosities on their finer twigs. They also enter into the plexus of the terminalis to some extent. They are given off from the fifth nerve. It is usually stated that the septal glands are innervated by the trigeminus, and these fibers appear to be the ones by which this is accomplished.

2. The nervus terminalis of the beef

The nervus terminalis of the beef, as shown in figure 32, lies median to the olfactory nerves, between the meninges and the ventral brain surface. Running parallel with it are branches of the anterior cerebral artery. In the specimen figured the greater portion of the left nerve is a compact bundle, while the right nerve is composed of several strands for the greater part of its length. In the numerous brains examined there was considerable variation in the relation of the different strands which by their union form the main nerve bundle. This variation was found not only when comparing one brain with another, but, as just indicated, on comparing the two nerves of the same specimen. In some cases the strands were independent up to a short distance from the forward margin of the hemispheres, in other cases the bundle was formed much further caudad.

For the greater part of its course the portion of the nerve present in the specimens was covered by the meningeal membranes. It emerges to the outer surface of the arachnoid coat at about the point where the cerebral hemispheres begin to curve upward. At this point the nerve was always compact in a single bundle.

This bundle after emerging lay free on the surface of the arachnoid. The appearance of the free end indicated that it had been stretched and broken in removing the brain from the cranial cavity.

Several ganglionic swellings are visible, the largest one in the brain from which the figure was drawn, at the point (fig. 32, *gn.*) where the nerve crosses the artery. Just caudad to this ganglion, as more clearly shown in figure 33, the bundle divides into two strands. A short distance rostral to this ganglionic

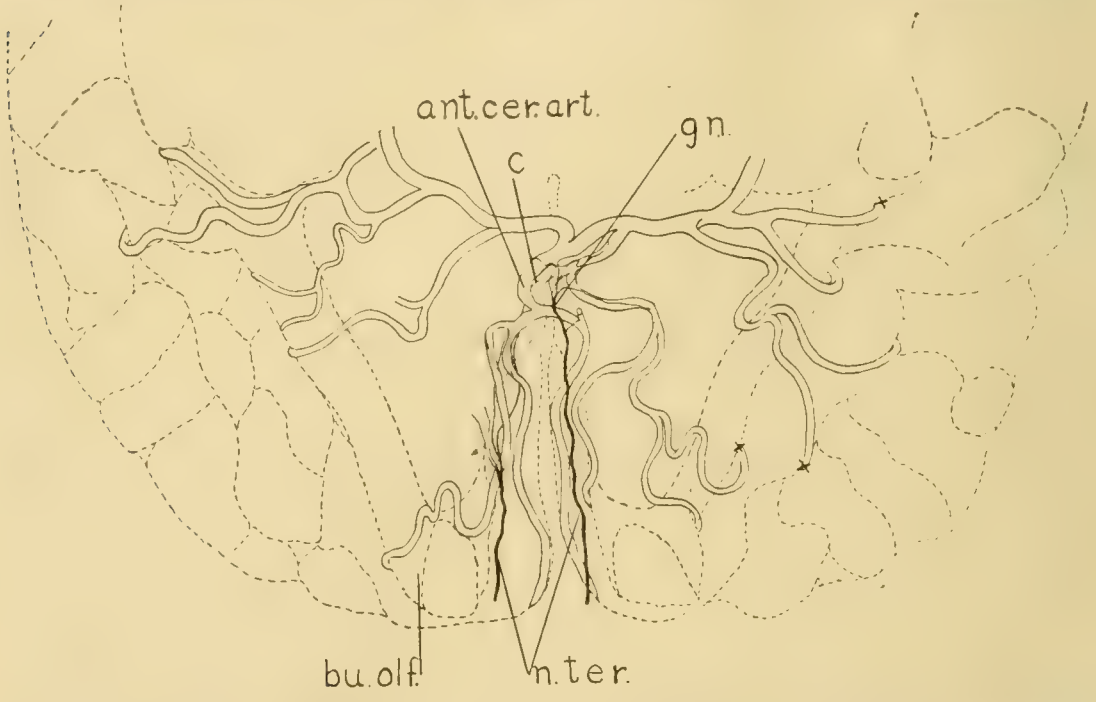


Fig. 32 Ventral view of anterior portion of the beef brain, showing the nervus terminalis and a portion of the anterior cerebral artery and some of its branches. Nerve strands traced along arteries to points marked *x*.

mass, a similar strand is given off from the main trunk. These strands follow the branches of the anterior cerebral artery, as indicated in figures 32 and 33, ramifying to the secondary branches of these vessels, and at intervals they give off fine twigs which penetrate into the muscular walls of the arteries.

Several of the strands were traced continuously to the points marked *x* in figure 32, where the respective arteries dipped into the fissures. On the same brain, and on others, similar strands were found on all of the arteries examined in this region of the



Fig. 33 Portion of left nervus terminalis and related vessels shown in figure 32, enlarged to show the distribution of strands to the walls of the blood-vessels.

brain, both on the ventral surface and in the sagittal fissure. In all cases in which their connection could be determined, when followed toward the ventral surface of the brain such strands led to the principal branches of the anterior cerebral artery, and connected with the main nerve bundle of the terminalis. When the overlying connective tissues were successfully removed the white nerve strands stood out clearly against the reddish brown of the blood-vessels. The larger bundles were followed with comparative ease even with the unaided eye, and were not easily torn. It is unlikely that all of the finer bundles were left intact. The relatively long stretches on some of the arteries shown in figure 33 where no twigs are represented probably indicate areas where they were inadvertently torn in dissection. Those shown in other parts of the vessels could be raised upon the point of a needle and stretched in such a manner as to clearly show that the finer twigs into which they ramify enter the walls of the blood-vessels.

Caudally, the principal roots which by their union form the nerve trunk, follow along the larger vessels as far as the latter could be traced without cutting into the brain. In most of the specimens examined the internal carotid artery had been severed so close to the brain in removing the organ that it was not possible to determine with certainty that any of it was present. There seems little doubt, however, that the continuation of strands from the main trunk of the terminalis unites with the plexus surrounding the internal carotid.

A branch from the main bundle was also followed along the posterior ramus of the anterior cerebral artery as far as the genu of the corpus callosum, and similarly on other rami of this vessel nerve strands were observed.

Evidence of direct connection with the brain was difficult to obtain. Delicate branches from the nerve strands on the arteries were found occasionally to enter openings in the anterior perforated space. These branches were extremely difficult to disentangle from the mass of small blood-vessels, connective tissue, and elastic fibers among which they were found. Many appeared to be related to the larger vessels which enter the brain

substance in this region. In the material examined, only one relatively large strand (fig. 32, *c*) was seen to enter the brain. This compared in size with the strands which enter the mule's brain (figs. 40, 41, 42).

Although the peripheral relations in the beef could not be determined in the available adult material, dissection of a number of ox fetuses of 110 mm. to 140 mm. greatest length brought

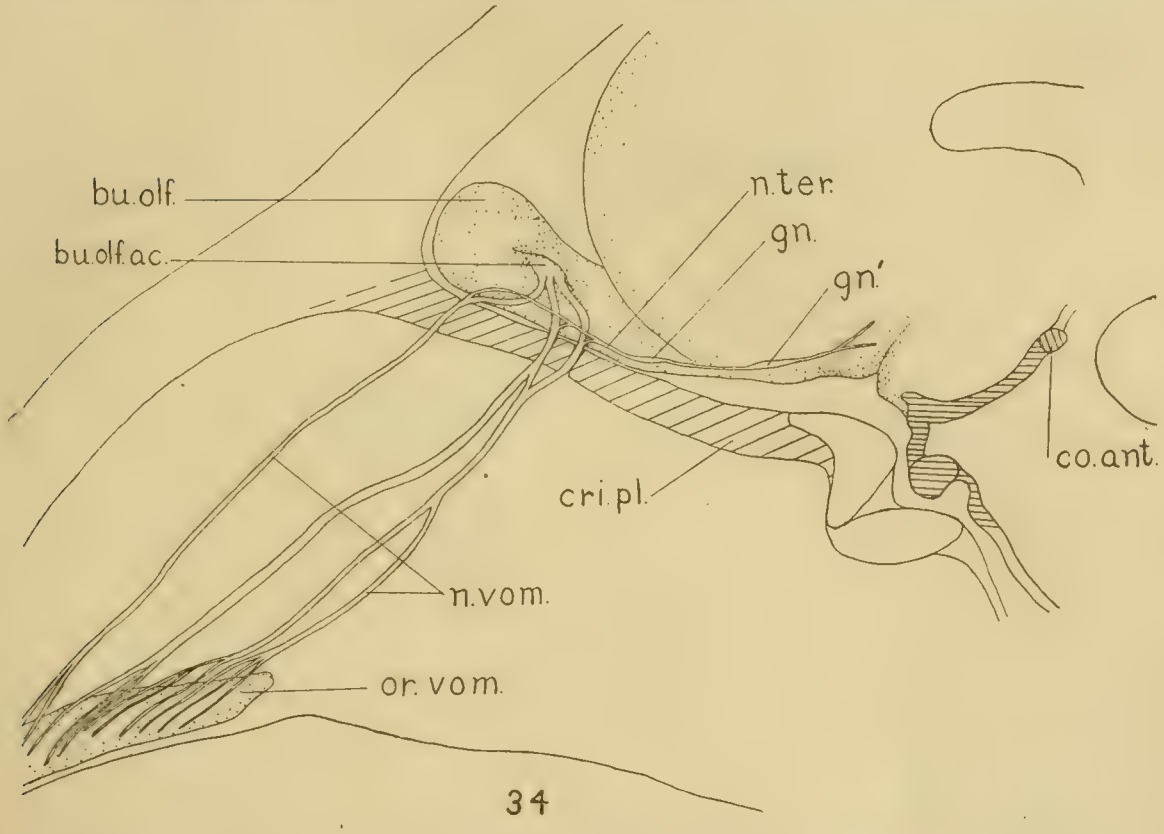


Fig. 34 Part of the nasal septum and the cerebral hemisphere of fetal ox of 121 mm. greatest length, showing the nervus terminalis, the vomeronasal nerves, and a portion of the vomeronasal organ. This drawing was combined from several dissections and is to that extent diagrammatic. × 4.

out the fact that in the beef, as in the other mammals studied, the terminalis passes through the cribriform plate in company with the vomeronasal nerve, and doubtless spreads out on the septum in the characteristic plexiform manner observed in other mammals.

As shown in figure 34, which represents the relations in a fetus of 121 mm. greatest length, the main nerve bundle divides

on approaching the cribriform plate into three strands, each of which unites with one of the bundles of the vomeronasal nerve. No differentiation between the terminalis and the vomeronasal nerve could be seen after the two had joined. Since they are separate in the cat, rabbit, horse, and human, there can be little doubt that differential staining methods would show the two as distinct in the septal region of the beef also. Attention may be directed at this point to the two ganglia which are visible on the

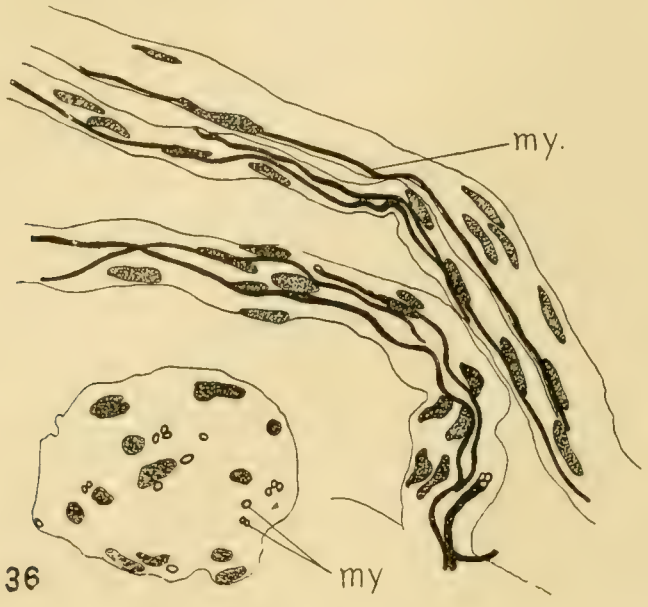


Fig. 35 Longitudinal section of two nerve strands accompanying anterior cerebral artery of the beef, showing myelinated fibers. Formalin fixation, iron-hematoxylin stain. Sections 10 μ. $\times$ 450.

Fig. 36 Transverse section of relatively large nerve strand parallel to anterior cerebral artery of beef, showing myelinated fibers. Formalin fixation, iron-haematoxylin stain. Sections 10 μ. $\times$ 450.

main central bundle of the nerve in the fetus and to the two roots at its central end. These roots appeared to enter the brain substance near the fissura prima.

Histological. The composition of the main nerve bundle in the adult, and of the principal strands which run parallel with the several branches of the anterior cerebral artery, was determined by the study of sections. As shown in figure 36, which represents a cross-section of one of the larger strands on the internal frontal branch of the anterior artery cerebral, sixteen

very delicate myelin sheaths are present, as brought out by iron-haematoxylin staining. More slender nerve strands in the same preparation showed a smaller number of myelinated fibers. In longitudinal sections (fig. 35) the myelin sheaths are shown to continue without interruption for considerable stretches. They measure from 1.5 μ to 2 μ in diameter.

Sections of the main trunk peripheral to the ganglionic masses revealed a much larger number of myelin sheaths. In the section illustrated (fig. 37) which was stained by the Weigert method, sixty-four delicate sheaths are visible. Attention may also be called in this connection to the many fasciculi, fifteen in number, which enter into the formation of the larger bundle.



Fig. 37 Transverse section of main trunk of nervus terminalis of beef, showing distribution of myelinated fibers. Formalin fixation, Weigert stain. × 360.

Great care was exercised to prevent decolorizing any of the sheaths during the process of differentiation, when those methods were employed which required caution. In view of the partial degeneration which had taken place in some of the sheaths, it is possible that this process had reached a stage in some fibers at which the staining methods employed were no longer effective in differentiating the myelin. It is believed, however, that the majority of myelinated fibers were stained. The remaining portion of the nerve strand was assumed to be made up of unmyelinated fibers. These observations were subsequently confirmed in material which was fixed in osmic acid shortly after the animal was slaughtered.

An attempt was made to analyze those roots which penetrate into the brain, using the osmic-acid material. The few roots which were successfully isolated were of small size. They were removed and mounted whole in glycerin. One or two myelin sheaths were observed in each case, the remainder of the root being evidently composed of unmyelinated fibers. They thus resemble, except in size, the strands on the arterial walls.

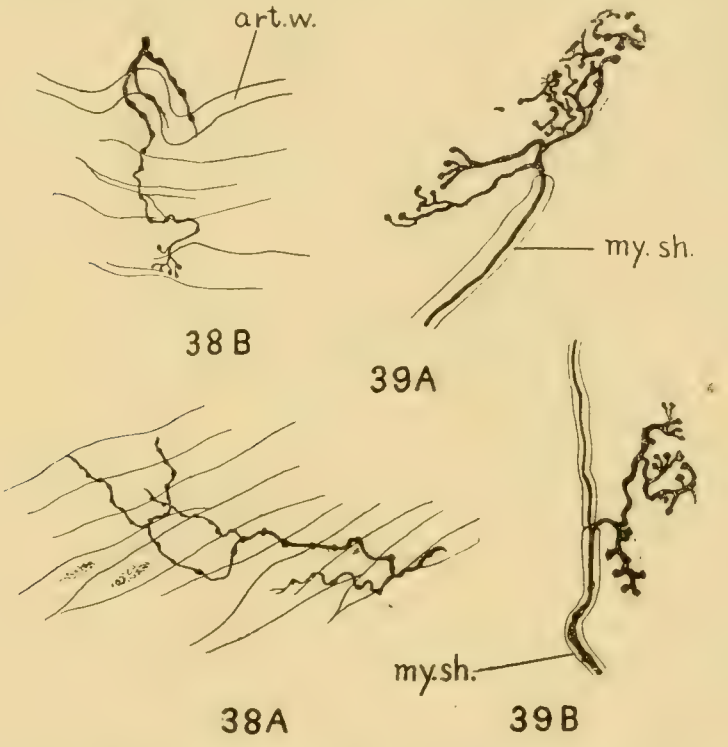


Fig. 38 A and B Motor terminations in muscular layer of anterior cerebral artery of beef. A illustrates the typical appearance, B showed slight terminal knobs. Gold-chloride technique. × 733.

Fig. 39 A and B Sensory nerve terminations in wall of anterior cerebral artery of beef, illustrating typical spindle-like outline and myelinated fibers. A represents a typical termination, B shows two smaller ones attached to a common branch. Gold-chloride technique. × 733.

Nerve terminations. The frozen beef brains responded well to the gold-chloride treatment, and terminations of the twigs which enter the muscular walls of the blood-vessels were found. These nerve endings, as in the cat, are of two types. Type I (fig. 38, A and B) consists of delicate varicosed fibers which ramify and run for varying distances among the unstriated muscle cells.

These fibers are unmyelinated, and correspond in appearance with those previously described in the cerebral vessels of the cat. They are present at various levels of the muscular coat of the arterial wall, as shown in cross-sections. They send their twigs between the smooth muscle cells in the typical manner of motor terminations in this type of muscle. As previously stated, it seems probable that they should be regarded as motor endings.

The terminations which have been designated as Type II are, as in the cat, strikingly different in appearance (fig. 39, A and B) from those of Type I. The nerve fiber leading to these is myelinated, as indicated by a reddish cylinder surrounding the purplish-black of the central axis. The myelin sheath in most cases continues almost to the spindle-shaped termination. The axis, after emerging from the sheath, divides into two or three main rami which give off a varying number of short processes. These processes terminate invariably in rounded knobs. The portion of the spindle-shaped organ which was not occupied by these processes appeared nearly clear or had a slight bluish tint and was slightly granular. It stood out in strong contrast to the purplish red of the surrounding muscle cells.

The spindles in the beef showed considerable variation in size. Of those measured, the smallest were 4 μ in diameter and 12 μ in length. The largest were 6 μ to 7 μ in diameter and 35 μ in length. The majority appeared to be about 5 μ by 25 μ to 30 μ. Figure 39 illustrates one of the largest observed (A) and also an example of the smallest size (B) drawn to the same scale. It will be noted that the two end-organs represented in figure 39 B are the terminations of what appeared to be a branch of the larger fiber shown in the figure. A node is seen in the myelin sheath, through which the branch to these terminal organs passes. In a few instances it was possible to follow the myelinated fiber to the external surface of the artery and for a little way beyond.

3. The nervus terminalis of the mule and the horse

The mule. As represented in figure 40, the nervus terminalis of the mule runs parallel to the olfactory tracts, between them and the sagittal fissure of the brain. The nerve in both the mule

and the horse, as in the beef, may be easily seen with the unaided eye. As shown in the figure, the left nerve had two compact ganglia. The right nerve had a single ganglionic mass of larger size. The larger of the two ganglia of the left nerve, which was the one more rostrally situated (figs. 40 and 43), was about 2 mm. long and $\frac{1}{2}$ mm. in diameter. The smaller ganglion was of about one-half the volume of the other.

The peripheral ends of both nerves were frayed and had the appearance of having been broken, doubtless where they subdivided into strands similar to those observed in the cat and in the horse. The anterior ends of the olfactory bulbs had been torn off in removing the brain from the cranial cavity, so it was not possible to study the relation of the terminalis to the vomeronasal nerves in this specimen. The main bundles of the nerve lay outside the pia mater as far caudally as several millimeters rostrad to the ganglia. Here they pierced the pia and in their further course centrally lay between this and the brain surface.

Immediately caudal to the posterior ganglion of the left nerve, and slightly more caudad to the single ganglion of the right nerve, the main bundle divides into a number of strands or rootlets. For convenience, the description will be confined to the left nerve, but in all essential respects, unless indicated to the contrary, the same statements apply to the right nerve also.

As shown in figures 40 and 43 the main trunk of the nerve is formed by the union of three strands. Two of these, the mesial one and the lateral (figs. 40 and 41), follow a course closely parallel to two of the branches of the anterior cerebral artery, and give off delicate rami to them. These twigs enter the walls of the vessels in the same manner as has already been described in the ox.

The relation of the medial of the three roots to another branch of the anterior cerebral artery is shown in figures 40 and 41 at *a*. Figure 40 represents the left hemisphere of the brain as viewed from the medial side. In figure 41 a portion of the same surface is represented on a larger scale, as seen through the binocular microscope. It will be noted that strand *a* turns so as to run nearly at right angles to the main nerve bundle and follows the

course of the artery. The nerve strand soon divides into two smaller strands, one of which had been inadvertently cut in separating the hemispheres. The severed portion may be seen on the stump of the artery, which it was ascertained belongs to the right hemisphere of the brain.

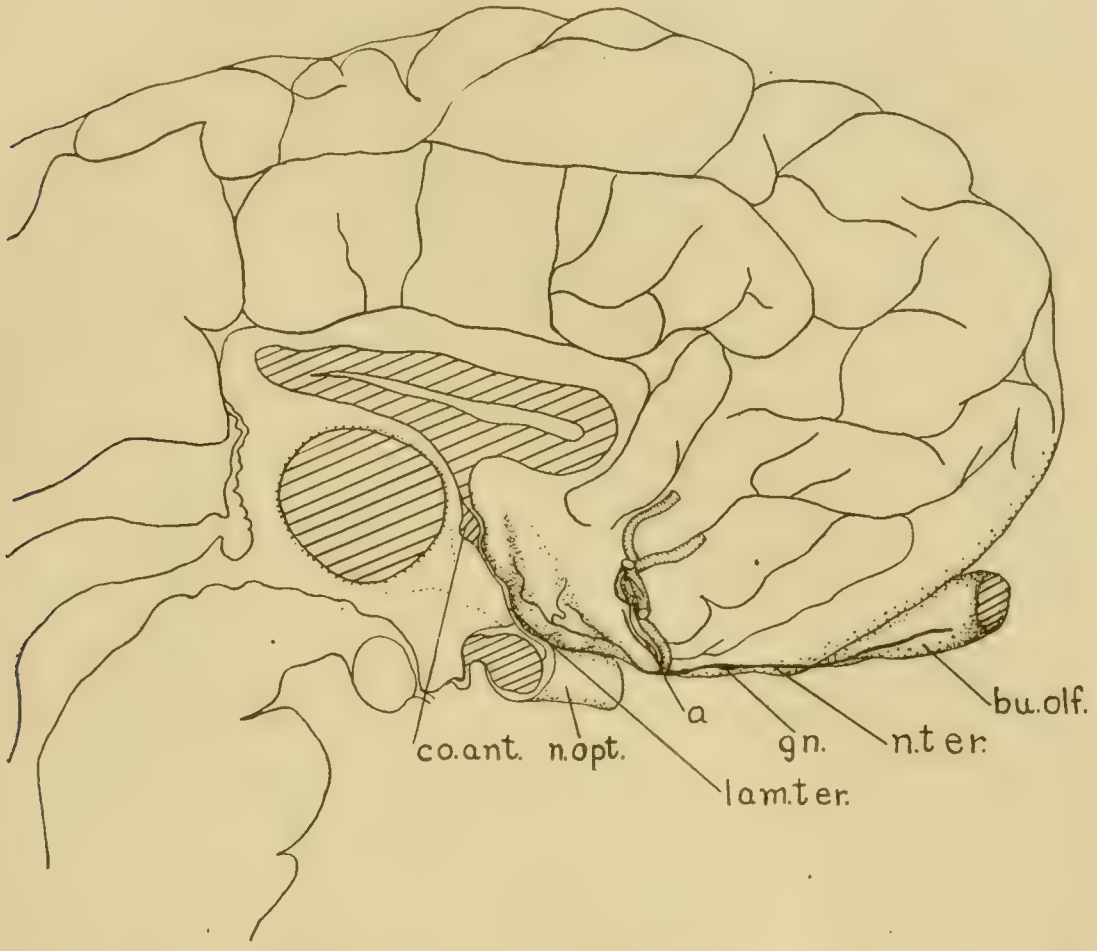
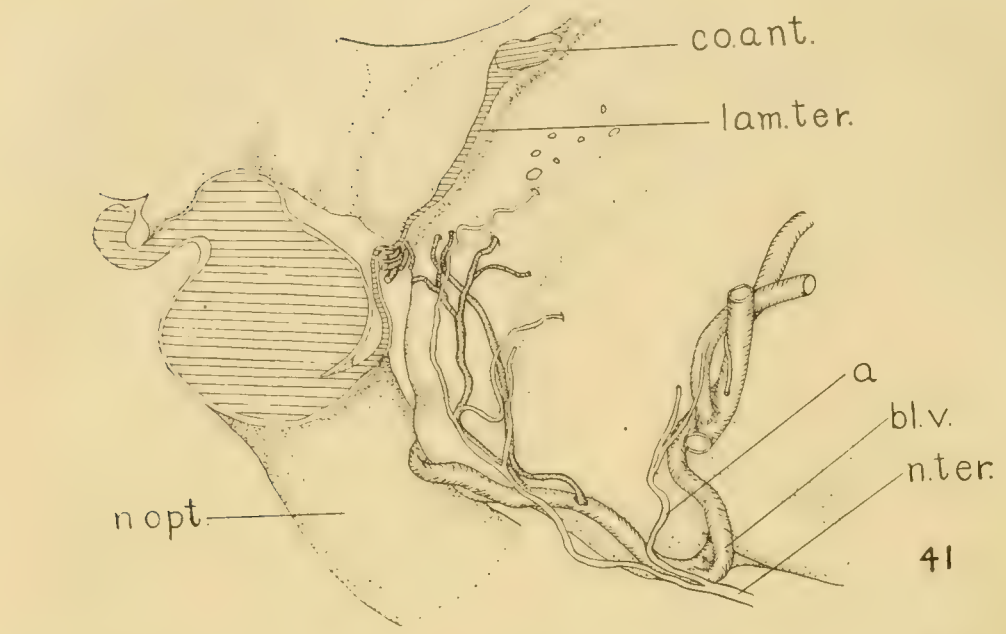


Fig. 40 Mesial view of left hemisphere of full-term mule fetus, showing the nervus terminalis. Slightly enlarged.

The middle root of the left nerve, as also shown in figures 40 and 41, runs toward the perforated area in front of the lamina terminalis. At first it is roughly parallel with the anterior cerebral artery, then turning upwards, it gives off a small ramus which enters the brain through a perforation in the furrow of the small sulcus (posterior parolfactory) shown in the figure. Another ramus of slightly larger size is given off a little further caudad. This, and the caudally continuing main strand, also enter the



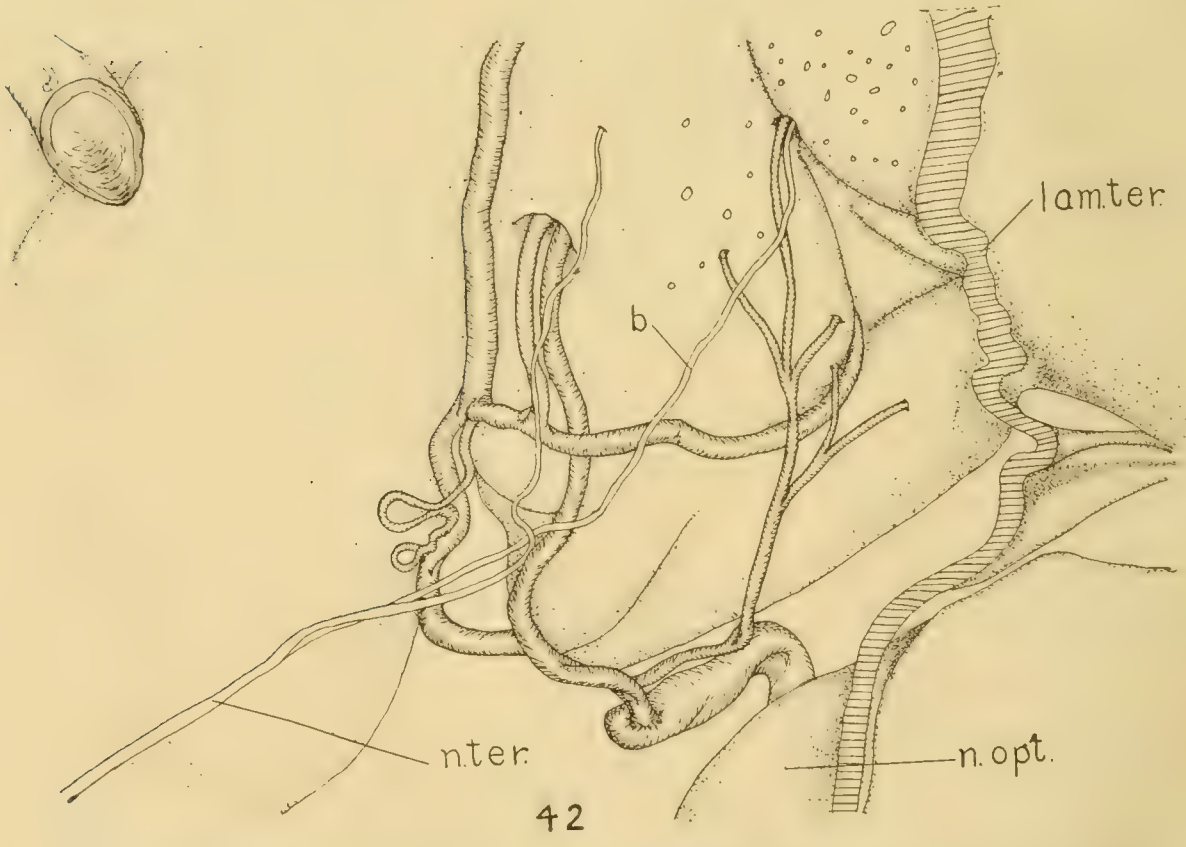


Fig. 41 Central connections of the left nervus terminalis of full-term mule fetus, illustrating relations to neighboring blood-vessels and points of entrance into brain substance. × ca. 5.

Fig. 42 Central relations of right nervus terminalis of mule fetus. × ca. 9.

brain substance, the one slightly rostrad to the sulcus previously mentioned, the other at a point about 2 mm. anterior to the lamina terminalis, and somewhat more than midway between the anterior commissure and the ventral border of the brain.

As already stated, essentially the same conditions are found on the right side of the same specimen (fig. 42). Here, however, only two roots were found which enter the brain substance. The larger one (*b*) enters through the same opening as does one of the blood-vessels of medium size. This was the only case observed in the mule where a nerve strand of the terminalis and a blood-vessel enter the brain together. Other rootlets enter very near the openings through which other blood-vessels pass. The main nerve trunks on both sides are very clearly formed by the union of the roots described, with the possible addition of other smaller ones which may have been torn and lost during the dissection.

The left nerve was removed from its attachments to make possible a closer study of its structure. As represented in figure 43, the three principal roots merge into the smaller ganglion. Rostrally from this ganglion the nerve is compactly enclosed in a sheath of connective tissue. A few millimeters rostrad from the small ganglion is the larger one previously noted. Continuing forward from this point, the nerve remains as a compact bundle as far as the place where it had been broken in removing the brain from the cranial cavity.

A very slender blood-vessel (fig. 43, *bl.v.*) winds around the nerve for the greater part of its course. At intervals this vessel gives off branches which penetrate into the nerve bundle.

Histological. Sections of the ganglia stained with thionin reveal a considerable variety in the form of the ganglion cells. The sections were cut 18 μ and 20 μ thick, so that the processes could be followed in many instances for some distance. As illustrated in figures 44 to 47, all variations of type from simple bipolar, to cells having at least five processes occur. Numerous binucleated cells (fig. 47) were observed. Of the 292 ganglion cells which were found in the single ganglion of the right nerve, twenty-seven were binucleated, or 9.25 per cent. Sections cut at 10 μ and stained with haematoxylin showed the same types of cells in the two ganglia of the left nerve.

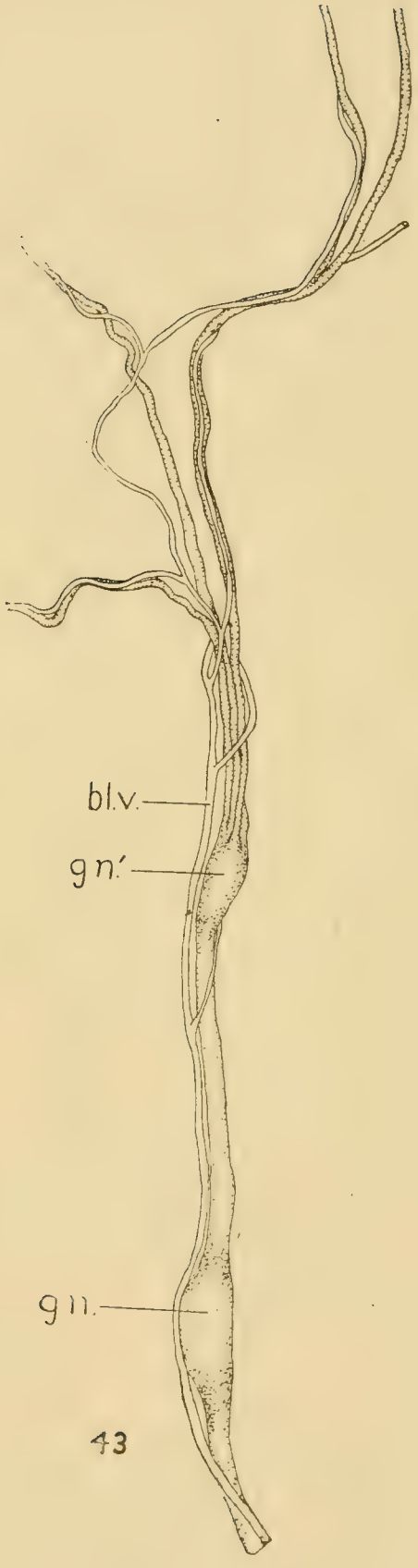


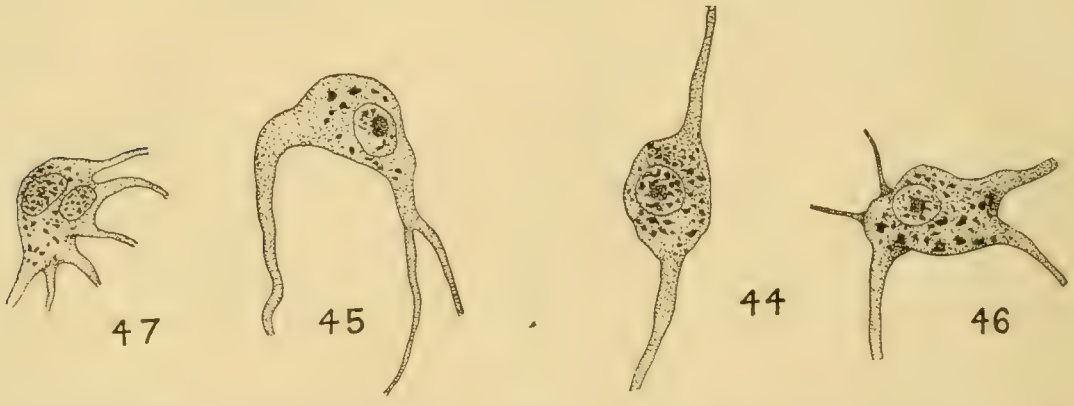
Fig. 43 Left nervus terminalis of mule fetus at full term, removed from its connections. Only the ganglia, with portions of the main trunk and of the central roots are represented.

Some attention was given to the arrangement of the chromatophile granules in the cells. Carpenter and Conel ('14) have pointed out the characteristic peripheral arrangement of this substance in ganglion cells of the sympathetic system, and hold this to be a distinguishing mark which differentiates them from cells of the cerebrospinal system. The granules are said to be scattered throughout the body of the perikaryon in ganglion cells of the central system. No very satisfactory results were obtained with the material available. Many of the cells (figs. 44 and 45) are seen to have a somewhat peripheral arrangement of the granules, while the other cells figured do not give sufficient indication of such distribution of granules to be noticeable. It should be stated, however, that the sections stained with thionin, which would affect the Nissl bodies, were too thick to be favorable for such a study.

An effort was made to demonstrate myelinated fibers, if present, and to learn, if possible in the large central roots of the terminalis of the mule, the relative number of such fibers to the number in the main peripheral trunk. The osmic acid, iron-haematoxylin, Weigert, and Stroebe methods were each tried, with variations, a number of times. No success was had in demonstrating myelin sheaths. In some of the preparations delicate fibers of small diameter were visible, but it was not possible to trace them continuously through more than three or four sections of a series. In the central roots from two to four such sheath-like structures were present in some of the sections. In others none could be seen. The peripheral bundle showed as many as sixteen in certain sections. The results of this part of the study were on the whole negative. Either the myelin sheaths are not well developed in the nervus terminalis at this stage of fetal life of the mule or the formalin preserved material did not respond to the methods employed for their demonstration.

The Horse. The nervus terminalis of the horse brain examined (fig. 48) consists centrally of four principal strands which unite near the base of the olfactory stalk to form a broad, flattened nerve trunk. This trunk continues rostrally as a compact bundle as far as the posterior part of the bulbus olfactorius, receiving

a number of delicate strands in this part of its course. Many of these strands were traced caudally to neighboring blood-vessels. The shrunken condition in which these vessels were found, however, did not favor an attempt to find twigs, such as were observed in the beef, which pass into their muscular walls. At the region where the olfactory stalk swells to form the bulbus olfactorius, the main trunk of the terminalis divides into five relatively large strands and a number of smaller ones. A few millimeters caudally of this point a single strand is given off, which appears to pass laterally and beneath the olfactory stalk. The five larger strands continue rostrally, anastomosing with the finer strands and with one another, and thus form a plexus very similar



Figs. 44–47 Ganglion cells from ganglion terminale of full-term mule fetus. Formalin fixation, thionin stain. × 550.

to that observed in the cat (figs. 1 and 3) and in the dog (fig. 49).

About 3 mm. rostrad to the point where these strands assume a separate course, and lying in the path of the largest of the five, is a large flattened ganglion (fig. 48. *gn.*) of irregular outline. This ganglion receives fibers also from other strands of the plexus than the one in whose apparent course it is placed, so that it lies in the midst of the plexus.

A much smaller ganglion (*gn'*,) is present caudally, on the main trunk midway between the larger ganglion and the point of union of the central roots.

Rostrally of the main ganglion, two of the larger strands enter the sheath of connective tissue which encloses the vomeronasal

nerve in its passage through the cribriform plate. One enters dorsally of the latter nerve trunk and the other on its ventral side. The vomeronasal nerve differs in the horse examined from the condition found in the other mammals studied in that it

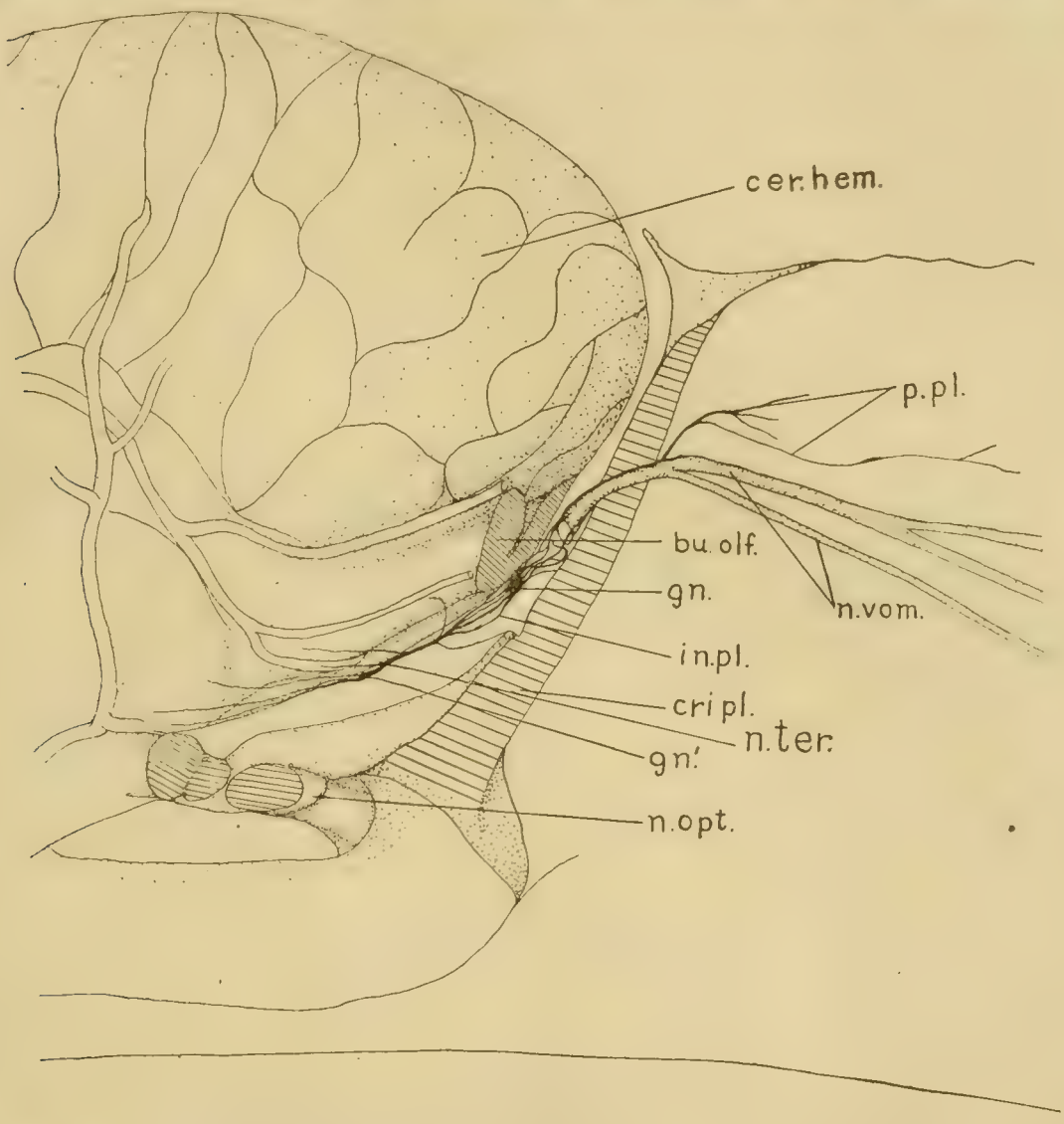


Fig. 48 Ventrolateral view of forward portion of the brain and of part of the nasal septum of the horse, showing the relations of the nervus terminalis. The olfactory bulb was removed to bring into view the ganglion terminale and the intracranial plexus, from the angle at which the dissection was made.

does not divide into the characteristic number of bundles until it has passed through the bony plate as a single compact trunk, surrounded by a heavy sheath. A relatively large strand of the terminalis penetrates this sheath immediately distal to the point

of emergence of the vomeronasal bundle from its foramen. This strand assumes an independent course, running dorsally a little way, then divides into a number of smaller strands whose general course is rostrally. These become so attenuated by repeated division that it was not possible to follow their finer ramifications by dissection. Apparently they form a portion of a plexus similar to that found in the cat and in other mammals.

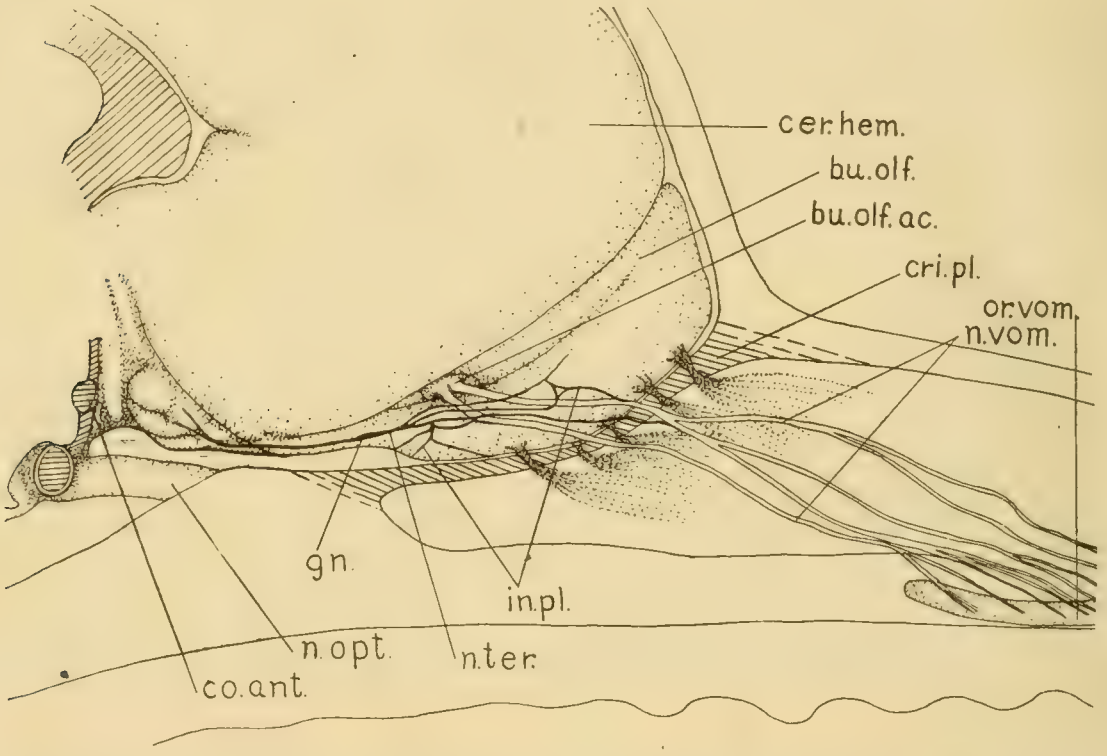


Fig. 49 Anterior portion of cerebral hemisphere and a portion of nasal septum of a puppy two weeks old (estimated) showing nervus terminalis.

The course of the nerve strands in the septum was followed to some extent by the expedient of stripping off the thick mucosa from the bony septum and examining its deeper surface. This required a minimum of dissection, as the nerve strands lay for the most part in the deeper portion of the mucosa. The proximal portions of the first two divisions of the vomeronasal nerve lay in rather deep furrows in the bony septum, and the strands of the terminalis which accompany the larger nerve through the cribriform plate, accordingly, emerge from the sheath very close to the periosteum of the bony septum. In addition to the rami

of the bundle just noted, other strands were observed in the septum which also appeared to form a part of the terminalis plexus.

Returning to the other intracranial strands which were noted distally of the ganglion terminale, the most dorsal one was somewhat torn in dissection, but appeared to have passed between the olfactory bulb and the cerebral hemisphere. The remaining fibers appear to pass through the cribriform plate in company with bundles of olfactory fibres, ventral to the vomeronasal foramen. Such fibers could not be found distally of the plate. Possibly the strands in the septum which were noted in connection with those which emerge in company with the nervus vomeronasalis are distal continuations of the strands now under discussion. The connections in the bony plate might easily have been inadvertently injured to such an extent that the strands immediately distal to the plate were completely destroyed.

4. The nervus terminalis in other mammals

The observations of the nervus terminalis of the dog, squirrel, and human, and in embryos of the pig, sheep, and rabbit, were less extended than those described in the preceding pages. So far as they were carried, they agreed with the findings in the other animals studied. The nerve was found in the typical relation to the vomeronasal bundles, and passes through the cribriform plate in company with these.

Only one ganglion was found in the dog. This is situated near the vomeronasal nerve, and is long and fusiform (fig. 49). In the squirrel also (not figured) one large ganglion was present at the point where the main trunk of the terminalis splits into strands similar to those found in the cat and the horse, which accompany the larger bundles of the vomeronasal nerve through the bony plate. A number of smaller strands, which appeared to correspond with the intracranial plexus found in the other animals, were observed. It may be noted that in the squirrel the accessory olfactory bulb lies on the dorsolateral side of the bulbus olfactorius so as to be invisible when the brain is viewed from the medial side.

As already stated, the attempt to find strands from the terminalis to the blood-vessels of the human brains was not successful. Nerve strands similar to those present on the anterior cerebral artery of the beef and the mule were found and small twigs were observed to enter the walls of the corresponding vessels of the human material. Efforts by the gold-chloride and Bielschowsky methods to demonstrate nerve terminations in these vessels were not satisfactory with the material available.

The studies on the embryonic material did not serve to reveal any features not already known.

III. SUMMARY AND COMMENTS

1. The nervus terminalis of mammals is made up in part, at least, of sympathetic fibers, and its ganglionic clusters contain sympathetic cells. The wide distribution and large number of fibers of the peripheral plexus (as noted by Brookover), in comparison with the small size of the central connections, resemble the relation of preganglionic fibers to the postganglionic fibers of the sympathetic system. This resemblance is strengthened by the occurrence of pericellular baskets on many of the ganglion cells of the intracranial clusters.

2. Two types of neurones are present in the terminalis, namely, 1) sensory and 2) motor.

3. Some of the sensory fibers end in the muscular walls of the anterior cerebral artery and its branches by a type of nerve termination hitherto undescribed in cerebral blood-vessels.

4. There is some evidence that free nerve terminations in the epithelium of the septal mucosa and of Jacobson's organ are also connected with afferent neurones of the nervus terminalis. For the present it is assumed that these free sensory terminations belong to a sensory component of the nervus terminalis which is distinct from sympathetic afferent fibers which have terminations in the walls of the blood-vessels.

The type of nerve endings and their position in the mucosa would seem to indicate that this component is part of the general visceral afferent system. The early embryonic history of the nerve in ganoids and selachians might, however, point to a rela-

tionship with the special visceral afferent group. As previously noted, Brookover states that the origin of the nervus terminalis in the ganoid fishes studied by him is from a portion of the olfactory placode. Locy, in describing its early development in Squalus, attributes its origin to the neural crest, but states also that "The new nerve has at first a fusion (placode) with the thickened surface epithelium, located just above the shallow depression that marks the beginning of the olfactory pit. This connection between the surface epithelium and brain-wall, consists of a group of closely packed cells in which I have failed at this early stage [6 to 8 mm.] to recognize fibers." If this placode described by Locy be homologous with that found by Brookover, the embryonic evidence in the two groups on which these writers worked points to an origin which in part at least corresponds to that of other special sensory ganglia in the head region.

In connection with the free nerve terminations described, this embryonic origin of the nerve from a placode suggests the conclusion that there is a sensory component of the terminalis which is distinct from the sensory fibers which terminate in the walls of the blood-vessels. The neuroblasts which have their origin in the neural crest might well give rise to the sympathetic cells described and figured in the present article, and which have been described in other groups of vertebrates than the mammals in the nerve under consideration. The free nerve terminations in the mucosa do not, however, seem to fit in with the view that this sensory component belongs to the special visceral system.

5. Many physiologists find insufficient evidence of vasomotor control of the cerebral blood-vessels. Wiggers ('05, '08) and Weber ('08) have presented experimental support of the histological evidence. Both conclude that there is direct physiological proof of nerve control over the cerebral vessels. Weber, moreover, finds indications that there is an accessory vasomotor center further rostrad in the brain than that situated in the bulb.

This observation is suggestive, in connection with McKibben's findings in urodeles, of terminalis tracts extending to the interpeduncular region, with a probable center in the neighborhood of the preoptic nucleus.

6. If connection of the nervus terminalis with such a center in the forward part of the brain should be demonstrated, the forebrain should be included in the list of those divisions of the central nervous system which are directly related to the sympathetic system.

7. The evidence now at hand points to the conclusion that the nervus terminalis of mammals is functional. Its relatively small size in this group as compared with its development in selachians, may indicate that its functional importance is reduced.

8. Innervation by the nervus terminalis of the vomeronasal organ, which appears to occur to some extent, is merely incidental.

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Resumido por el autor, Edward Phelps Allis Jr.

Los nervios oftálmicos de los peces gnatostomos.

En Polypterus sale del cráneo, con las fibras del lateral que van a los nervios oftálmico superficial y bucal lateral, un fascículo de fibras del común, formándose un ganglio con estos dos fascículos, colocado en posición dorsal con relación al ganglio cutáneo general del trigémino. De este ganglio común-lateral parten fibras del común, las cuales van a parar al ganglio cutáneo general, que no envia fibra alguna al primero. El oftálmico superficial se origina en su totalidad en el ganglio lateral común y contiene fibras del lateral y común, pero en pequeño número, careciendo probablemente de fibras del cutáneo general. El llamado trigémino-oftálmico profundo es en realidad un ramo oftálmico profundo puesto que nace de un ganglio profundo e independiente, formado por una raiz profunda independiente; aparentemente contiene solo fibras cutáneas generales. Posee ramas frontales y nasales que son homólogas de las mismas ramas del nervio oftálmico de los vertebrados más superiores. Este último nervio es también por esta causa un nervio profundo y no una parte del trigémino. En los Dipnoos la distribución es, en apariencia, las misma que en Polypterus. En los Holósteos hay un ramo oftálmico superficial que contiene fibras del lateral, común y cutáneo general y una porción oftálmica profunda, pero el ramo oftálmico profundo, cuando existe, está degenerado. En ciertos Teleósteos (Gasterosteus) existe una distribución semejante a la mencionada en los Holósteos, pero en la mayor parte de ellos hay un ramo oftálmico superficial de composicion variable, faltando el ramo oftálmico y la porción oftálmica profunda.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, DECEMBER 9

THE OPHTHALMIC NERVES OF THE GNATHOSTOME FISHES

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There has long been, and still is, confusion in the terms employed to designate the ophthalmic nerves of the gnathostome fishes. These nerves were formerly considered to be the equivalents of the ramus ophthalmicus trigemini of higher vertebrates (Stannius, '49), and as in many fishes, one of them lies superficial to the other they were called the rami superficialis and profundus trigemini. The term profundus was, however, frequently given to two distinctly different nerves, one of which runs forward between the superior and inferior divisions of the nervus oculomotorius and then ventral to the nervus trochlearis, while the other runs forward dorsal to both those nerves. The former nerve is alone properly called a profundus and it is typically found in the Elasmobranchii. A nerve that, in certain fishes, connects the basal portion of this profundus nerve with that of the superficialis was called the portio ophthalmici profundi.

Later, it was found that those nerve fibers of the superficialis nerve that innervate the organs of the supraorbital laterosensory line all issue from the medulla as an apparent part of the root of the nervus facialis, and as this was considered to be incontrovertible evidence that they belonged to the latter nerve, that part of the ophthalmicus superficialis that was formed by them was called the ramus ophthalmicus superficialis facialis. The remaining fibers of the superficialis nerve were still considered to belong to the trigeminus, and were usually called the ramus ophthalmicus superficialis trigemini, but as, in the Holostei and Teleostei, they lie deeper than the lateralis fibers, they were frequently called the ramus ophthalmicus profundus trigemini, and

apparently considered to be the homologue of the similarly named nerve of the Elasmobranchii. This latter nerve of the Elasmobranchii was still called the ramus ophthalmicus profundus trigemini, but there was a growing opinion that it belonged to a cranial segment next anterior to that of the trigeminus.

It was then still later found that communis fibers might also form part of the ophthalmicus superficialis, and that, like the lateralis fibers of that nerve, they issued from the medulla as an apparent part of the root of the nervus facialis. Consistency then evidently demanded that these communis fibers also be included in the ophthalmicus superficialis facialis, but as that term had come to mean a purely lateralis nerve, the communis fibers were either still relegated to the ophthalmicus superficialis trigemini or a new term, truncus supraorbitalis, was given to the entire ophthalmic nerve, the term ophthalmicus superficialis facialis still being employed to designate the lateralis fibers only of the nerve.

The ramus ophthalmicus superficialis of fishes thus came to be considered to be a nerve formed by the secondary juxtaposition of fibers derived from two adjacent segmental nerves, the general cutaneous fibers of the nerve being derived from the nervus trigeminus and the lateralis and communis fibers from the nervus facialis. This schema of the nerve still, however, left unaccounted for those fibers that were known to be derived, in certain fishes, from the portio ophthalmici profundi, and although this portio, as an independent nerve, had only been described in a few fishes, there was no apparent reason for assuming that it did not also exist in many, if not all, of those fishes in which the profundus and trigeminus ganglia have completely fused with each other.

This confusion of terms and complication of conditions led me, in my work on the mail-cheeked fishes (Allis, '09) to readopt the term, ophthalmicus superficialis trigemini, first given to this nerve, and to call its lateralis and communis fibers the lateralis and communis trigemini. A name of some sort had to be given to the nerve, and this one seemed to me to be the "single name already current" that Herrick ('09) has later suggested should be

selected for each so-called composite nerve, and it certainly had valid claim to priority over the one that he had himself earlier employed, namely, truncus supraorbitalis. Furthermore, it was at that time, and still is, my opinion that it has by no means as yet been definitely established that the lateralis and communis fibers found in the ophthalmicus superficialis do not belong definitely to the trigeminus, their centers of origin having simply fused with, or become contiguous to, those of similar fibers that belong to the nervus facialis. The conditions in Polypterus, described immediately below, certainly favor this conclusion, but they at the same time further complicate the choice of a proper name for the nerve.

In Polypterus there is a profundus ganglion which, in a 75-mm. specimen examined in serial transverse sections, is extracranial in position and lies wholly anterior to, and independent of, the trigeminus ganglion. The root of this ganglion traverses a foramen that lies anterior to the foramina for the roots of the nervus trigeminus, enters the medulla slightly anterior to the general cutaneous root of the latter nerve, and is, so far as can be determined from my somewhat imperfect and unsatisfactory sections, composed exclusively of general cutaneous fibers. From this profundus ganglion a typical ramus ophthalmicus profundus arises, and also either a single nerve, which immediately bifurcates, or two independent nerves, these latter one or two nerves forming the portio ophthalmici profundi shown by van Wijhe ('82) in his figure of this fish. The branches of this ramus and portio all join the ramus ophthalmicus superficialis, and, accompanying it and its branches, but in no way fusing with them, are distributed mainly to tissues on the dorsal surface of the anterior portion of the head. The ramus ophthalmicus superficialis arises from a ganglion formed on two bundles of fibers, one of which contains all the lateralis fibers that go to the rami ophthalmicus and buccalis lateralis, while the other is an intracranial branch of the communis root of the nervus facialis. These two roots of this ganglion, which thus quite certainly contain no general cutaneous fibers, issue together from the cavum cerebrale cranii, and the ganglion formed on them lies dorsal to the ganglion

formed on the general cutaneous root of the trigeminus. Communis fibers are sent from this lateralis-communis ganglion to the general cutaneous one, but no fibers can be traced from the latter to the former ganglion. The ramus ophthalmicus superficialis, which arises wholly from the lateralis communis ganglion and contains both lateralis and communis fibers, thus certainly contains but few, and probably no general cutaneous ones. The ophthalmicus profundus must then supply most, and probably all, of the general cutaneous fibers that go to that part of the dorsal surface of the head that is supplied, in most teleosts, exclusively by branches of the so-called ophthalmicus superficialis trigemini, and the latter nerve is wholly wanting in Polypterus.

The ophthalmic nerves of Polypterus thus are: a nerve that I should call the ramus ophthalmicus superficialis trigemini, but which, according to currently accepted views, would be called the ramus ophthalmicus superficialis facialis; and a ramus ophthalmicus profundi, which has ramuli frontalis and nasalis that are, respectively, the portio ophthalmici profundi and ramus ophthalmicus profundus trigemini of current descriptions. If the ophthalmicus superficialis were to abort, as the fibers that form it always do in all land vertebrates, there would remain a nerve that would quite unquestionably be the homologue of the ophthalmic nerve of Hoffmann's ('86) descriptions of embryos of reptiles, for the radix longa of my 75-mm. Polypterus, which arises from the profundus ganglion, is the ramus ciliaris of Hoffmann's descriptions of reptiles, which latter nerve fuses, for a certain distance, with the ramus nasalis to form the ramus nasociliaris. The opinion long ago expressed by His ('87, p. 398), that the ramus nasalis, or nasociliaris, of mammals corresponds to the so-called ramus ophthalmicus profundus trigemini of selachians is thus confirmed, as is also my conclusion, in my work on Mustelus (Allis, '01, p. 299), that the ramus ophthalmicus profundus of Polypterus and the portio ophthalmici profundi of ganoids are, respectively, the homologues of the nasal and frontal branches of the ophthalmic nerves of higher vertebrates. There would then be no so-called ramus ophthalmicus superficialis tri-

gemini in these latter vertebrates, which is in accord with Pinkus's ('94) conclusion that the presence of this nerve in the Amphibia is very doubtful, and with Norris's statement ('13, p. 292) that, in Siren lacertina: "It is questionable whether any general cutaneous fiber should be considered as a constituent part of the dorsal, or supraorbital division [of the truncus supraorbitalis] of Siren." The ramus ophthalmicus profundi of Polypterus thus quite certainly being the homologue of the so-called ophthalmicus trigemini of man, the introduction of a proper and uniform terminology becomes a somewhat radical proceeding, for it evidently requires a renaming of the nerve in man.

The conditions in those fishes, other than Polypterus, in which either a ramus ophthalmicus profundus trigemini, or a portio ophthalmici profundi, has been described, may now be considered, and I have, furthermore, examined the conditions in Gasterosteus, Cottus, and Clinocottus, in which fishes there is an anterior portion of the ascending process of the parasphenoid that occupies the position of the pedicel of the alisphenoid of Amia. The reason for examining these latter fishes was the conviction that, if there were a portio ophthalmici profundi, it would lie anterior to the above-mentioned anterior process of the parasphenoid, for that is the relation that the radix profundi of these fishes has to that process; and, conversely, if no branch of the profundus, sent to the superficialis, were found in that position, it would be, in my opinion, conclusive evidence that the portio ophthalmici profundi was wanting in these fishes, and hence presumptive evidence that it was also wanting in those of the Teleostei in which the process is not found.

In Protopterus, Pinkus ('94) describes a ramus ophthalmicus profundus trigemini, but no portio ophthalmici profundi. The first three branches of the ophthalmicus profundus are small, and, running forward dorsal to the nervi oculomotorius and trochlearis, become associated with a ramus ophthalmicus superficialis facialis. The ophthalmicus profundus then separates into two nearly equal portions, one of which is shown, in the figure given, running forward dorsal to both divisions of the nervus oculomotorius, and the other ventral to them, the dorsal branch then

apparently passing ventral to the nervus trochlearis and joining the first three small branches of the nerve. The relations of this profundus nerve to the oculomotorius thus differ radically from those in Polypterus, but it seems probable that its first three branches correspond to the frontal branch of the nerve of the latter fish, and the two larger ones to the nasal branch. A small nerve is said to arise from that part of the trigeminus ganglion from which the ophthalmicus profundus has its origin, and to immediately join the ophthalmicus superficialis facialis, and Pinkus doubtfully calls it the ramus ophthalmicus superficialis trigemini. Comparison with Polypterus would, however, indicate that it is a persisting remnant of the communis component only of the ramus ophthalmicus superficialis of the latter fish.

In Ceratodus there is a bar of cartilage that represents the pedicel of the alisphenoid (Allis, '14), and the ophthalmicus profundus of Greil's ('13) descriptions of embryos of this fish issues from the cranium anterior to that bar. The nerve is then shown, in one of Greil's figures (l.c., fig. 8, pl. 55), separating into two branches, one of which is evidently a portio ophthalmici profundi and the other a typical so-called ophthalmicus profundus trigemini. The ophthalmicus superficialis of this figure, called by Greil the ophthalmicus superficialis trigemini in another figure (fig. 4) on the same plate, receives no branch from what is apparently the general cutaneous ganglion of the trigeminus, the profundus thus supplying, as in Polypterus, all the general cutaneous fibers sent to the dorsal surface of the anterior portion of the head.

In Amia I have fully described the nerves here concerned, without, however, definitely determining their components (Allis, '97). It is, however, probable that the ophthalmicus superficialis contains lateralis, communis and general cutaneous fibers, and it receives an important portio ophthalmici profundi from an independent profundus ganglion, the root of the latter ganglion issuing from the cranium anterior to the pedicel of the alisphenoid. A delicate nerve that arises from the anterior end of the profundus ganglion was considered by me to be a greatly degenerated ramus ophthalmicus profundus.

In Lepidosteus, van Wijhe ('82) describes both a ramus ophthalmicus profundus trigemini and a ramus ophthalmicus superficialis trigemini. In a 75-mm. specimen of this fish I find the so-called ophthalmicus superficialis trigemini composed of lateralis, communis and general cutaneous fibers, the lateralis fibers forming a bundle which lies somewhat above the communis and general cutaneous ones. A radix profundi arises from the medulla anterior, and close to the general cutaneous root of the trigeminus, and issues from the cranial cavity by an independent foramen. A profundus ganglion forms on this root, and from it a radix longa, a ramus ciliaris longa, and a portio ophthalmici profundi arise. There is no perceptible trace of a ramus ophthalmicus profundus. The portio ophthalmici profundi runs upward and joins and fuses with the communis and general cutaneous components of the ophthalmicus superficialis, as shown but not index-lettered in Luther's figure of this fish ('13, fig. 1), but as there is no pedicel to the alisphenoid of this fish (Allis, '09) the relations of the nerve to that element of the skull are, as in Polypterus, undefined. The conditions in this embryo thus show that the so-called nervus ophthalmicus profundus of Landacre's ('12) descriptions of a 10–mm. embryo of this fish is probably a portio ophthalmici profundi and not a ramus profundus; and this nerve accordingly cannot be, as Landacre concludes in a later work ('16, p. 27), a nerve comparable to the ramus ophthalmicus profundus of the Selachii.

In Acipenser van Wijhe ('82) describes a so-called ophthalmicus profundus trigemini, but as this nerve is said to run forward dorsal to all the muscles of the eyeball, and is shown in his figure lying dorsal to the nervus trochlearis, it must be either a portio ophthalmici profundi, a ramus ophthalmicus superficialis trigemini, or both those nerves combined. Which one it is cannot be told either from his descriptions or from those of Gorono-witsch ('83), for the profundus ganglion is completely fused with the trigeminus ganglion.

In a 40-mm. specimen of Gasterosteus acus, in which fish there is an anterior portion of the ascending process of the parasphenoid that replaces the pedicel of the alisphenoid (Allis, in press), the

ophthalmic and maxillomandibular branches of the trigeminus issue from the cranium posterior to that process, but two small branches that arise from the intracranial portion of the trigeminus ganglion run forward in the cranial cavity and issue from it anterior to the process. One of these two branches is the radix longa. The other separates into two parts, one of which is the ramus ciliaris longa and the other a portio ophthalmici profundi. In a 37-mm. specimen of Cottus aspera, and in a 40-mm. specimen of Clinocottus, in both of which fishes there is also a process of the parasphenoid that replaces the pedicel of the alisphenoid (Allis, '09), there is a radix profundi which issues from the cranium anterior to that process and separates into a radix longa and ramus ciliaris longa, but there is neither portio ophthalmici profundi nor ramus ophthalmicus profundus. In each of these three fishes the profundus ganglion is so completely fused with the trigeminus ganglion that, while its general outlines can be readily determined, it cannot be told whether or not there is any exchange of fibers between the two ganglia. The conditions nevertheless show that where there is both a portio ophthalmici profundi and a process of the parasphenoid that corresponds to the pedicel of the alisphenoid, the portio issues from the cranium anterior to that process. Where there is neither pedicel of the alisphenoid nor corresponding process of the parasphenoid, as in most of the Teleostei, it might be assumed, as already stated, that the portio still existed, but the conditions in the three fishes above described, and those in Scomber (Allis, '03), where there is an independent profundus ganglion, but neither portio profundi nor ramus ophthalmicus profundus, tend to show that both these nerves have wholly disappeared in most of the Teleostei.

In a 22-mm. embryo of Squalus acanthias, Landacre ('16) finds both a so-called ramus ophthalmicus profundus and a general cutaneous nerve which he considers to be the ramus ophthalmicus superficialis trigemini, but the manner of origin of this latter nerve from the trigeminus ganglion is not incompatible with its being a portio ophthalmici profundi. There is, however, an anterior prolongation of the profundus ganglion that

strongly suggests this portio. Of this process Landacre says that it "is evidently the remains of the structure which Neal identifies as a persistent connection of the ganglion with the ectoderm, and which Scammon identifies as the utrochlea process, i.e., the remains of the connection of this ganglion with the neural crest."

The conditions in the several fishes above considered thus show that there are two distinctly different nerves that may supply the general cutaneous fibers that are distributed to the dorsal surface of the anterior portion of the head. One of these nerves is the so-called ramus ophthalmicus superficialis trigemini, the other what I have called the ramus ophthalmicus profundi, the frontal branch of the latter nerve being the so-called portio ophthalmici profundi. The trigeminus one of these two nerves always issues from the cranium posterior to the pedicel of the alisphenoid, or posterior to a corresponding process of the parasphenoid, while the profundus always issues anterior to that pedicel or process, and I consider these peripheral relations to these structural elements to be as definite and positive evidence of the segments to which the nerves belong as are the facts of development and the central origins of the nerves.

The relative importance of these two ophthalmic nerves varies greatly in different fishes, as does also the relative importance of the frontal and nasal branches of the ramus ophthalmicus profundi, and it would seem as if the frontal branch alone of the latter nerve might be the serial homologue of the entire ophthalmicus trigemini. In the Selachii, where there is apparently both a ramus ophthalmicus profundi and a ramus ophthalmicus trigemini, the portio ophthalmici profundi is wholly wanting, unless it be represented in some part of the so-called ophthalmicus trigemini. In the Holostei and certain of the Teleostei there is an ophthalmicus trigemini and a portio profundi, but the ramus ophthalmicus profundi, if present at all, is a small and degenerate nerve (Amia). In certain others of the Teleostei (Scomber) there is an ophthalmicus trigemini, but neither ramus ophthalmicus profundi nor portio profundi. In Polypterus, and probably also in Ceratodus, there is a ramus ophthalmicus pro-

fundi, with ramuli nasalis and frontalis, but no evident trace of an ophthalmicus trigemini, excepting as it is represented in the so-called ophthalmicus superficialis facialis; and this, with the further absence of the ophthalmicus superficialis facialis, is apparently the condition found in all higher vertebrates.

Herrick ('09) says that the facialis nerve of primitive vertebrates was a branchiomeric nerve, supplying a gill-bearing segment and containing at least four components. It is not said that one of these components was a general cutaneous one, but that seems understood. In the mandibular arch similar conditions must certainly have existed, and probably also in a premandibular arch supplied by the nervus profundus. In any event, it is certain that the general cutaneous tissues of the region innervated in Polypterus, Ceratodus, and the Selachii by the nervus profundus are innervated, in the Holostei and Teleostei, either by both the portio profundi and the ophthalmicus trigemini, or by the latter nerve alone. There must then have been, phylogenetically, either an actual change of innervation of these tissues in one of these two groups of fishes or the tissues innervated, in one of the groups, by one segmental nerve, must have degenerated, with the related nerve, in the other group, and there have been replaced by tissues primarily innervated by the nerve of an adjacent segment. The presence, in Amia, of a degenerate ramus ophthalmicus profundi would seem to exclude the possibility of assuming that the remaining fibers of the latter nerve have simply, in the Holostei and Teleostei, acquired a different and more favorable course than that followed by them in the Selachii. But however this may have been, it is evident that, of the several fishes above considered, Polypterus, the Dipneusti, and the Elasmobranchii alone present actual conditions of these nerves that could have led to those found in higher vertebrates, for that a nerve once so degenerated as the ramus ophthalmicus profundi of the Holostei and Teleostei would have been redeveloped and perpetuated seems improbable.

Palais de Carnolès, Menton, France
August 26, 1918.

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Resumido por el autor, Darmon Artelle Rhinehart.

El nervio facial del ratón albino.

La porcion motriz del nervio facial del ratón de distribuye sobre la musculatura facial e hioidea. El nervio intermedio se compone de fibras aferentes y eferentes, y una parte de las últimas forma una raiz separada. El ganglio geniculado contiene los cuerpos celulares de las fibras aferentes, perteneciendo estas células al tipo bipolar. El nervio petroso superficial mayor lleva fibras aferentes y eferentes al ganglio esfenopalatino, que no recibe fibra alguna del trigémino; sus fibras terminan en la glándula de Stenson, glándula lagrimal, glándula del tabique y vasos sanguineos de la nariz y en el paladar. El autor no ha podido determinar exactamente la inervación de las glándulas palatinas y botones gustativos. La cuerda del tímpano envia fibras a los ganglios de las glándulas submaxilar y sublingual y termina en la lengua; lleva las fibras gustatorias de los dos tercios anteriores de dicho órgano y probablemente desempeña otras funciones. El autor halló cerca de mil células ganglionares, de relaciones y función desconocida, en el trayecto de los nervios de una de las mitades de la lengua. Las fibras cutáneas procedentes del ganglio geniculado terminan en la piel del meato auditivo externo, piel de la oreja, y es posible también que inerven parte de la membrana del tímpano; estas fibras forman el ramo cutáneo-facial, rama independiente del facial. Las fibras del ramo auricular del vago forman una parte de este nervio y fibras del cutáneo-facial se distribuyen por el ramo auricular del vago. El nervio petrosomenor superficial falta en el ratón.

Translation by Dr. José Nonidez,
Columbia University.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 2

THE NERVUS FACIALIS OF THE ALBINO MOUSE

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FOURTEEN FIGURES

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INTRODUCTION

Almost all anatomical researches on the nervus facialis of man and larger mammals have been done on material which has been removed from the interior of the temporal-bone, a procedure which results in the destruction of important relations and only

gives pieces of the nerve for study. To a certain extent, this difficulty probably accounts for some of the conflicting statements as to the distribution and function of the nerve in these forms. In lower vertebrates, in which it has usually been followed in its entire course in serial sections through the heads of small animals, the study of the nervus facialis has given far more uniform results.

After a futile attempt to obtain satisfactory material from man and certain of the larger mammals for an investigation of the mammalian facial nerve, it occurred to me that a better procedure would be to follow the method used so successfully on lower vertebrates and select for use a small mammal, the head of which could be cut in serial sections. Because of the abundance of the material and because the size of the head readily permitted of the preparation of serial sections, the albino mouse was chosen for this work.

MATERIALS AND METHODS

The material used consisted of a number of series of sections of whole and half heads of 14-, 21-, and 23-day-old albino mice, mice of this age being selected because of the small size of the head and because the bones could be easily decalcified. The series were prepared by the pyridine silver technique following decalcification as originally used by Huber and Guild ('13 a). The original method was varied in that the vessels were first washed out with normal salt solution, and the stained material was imbedded in both celloidin and paraffin. The double imbedding was found necessary because of the small pieces of hair which were scattered over the sections when the blocks are imbedded in paraffin alone.

This modification of the pyridine silver method gives excellent results. The main trunks and branches of the nerves, nerve fibers in muscle and connective tissue, the ganglion cells on the roots of the cranial nerves and the cells and fibers in the central nervous system are well impregnated. The nerves in the glands and the cells in the sympathetic ganglia are the poorest stained of any parts of the cranial nervous system. In the sym-

pathetic ganglia there is an abundance of very fine fibers, which, together with the thickness of the sections, is responsible for obscuring the individual cells.

The heads were cut from the body in the lower cervical region, and in most instances were cut in halves following the second treatment with ammoniated alcohol. These smaller pieces were much more easily handled and gave more satisfactory sections than the heads which were carried through whole. Sections were cut 15 μ in thickness and mounted in series. Six sagittal series, six transverse series, and two horizontal series of half heads, and one horizontal series of a whole head were used in this work.

The method used in following the nervus facialis varied with the particular part under consideration. Perhaps the most useful consisted of making outline drawings of each section or of sections at regular intervals and tracing the different parts of the nerve from drawing to drawing. Another valuable method was a graphic method recently devised by Prof. A. G. Pohlman and explained to me during a visit to his laboratory. This method was less time consuming than profile reconstructing and gave results easy of interpretation. By projecting sagittal sections onto a median plane, flat reconstructions of parts of the nerve were made. In a few instances, to bring out important details, blotting-paper models were made.

In order to determine as far as possible the distribution of certain branches of the facial nerve, it was necessary to undertake and record a study of the nerve supply to parts of the head, namely, the sphenopalatine ganglion and the nerves connected with it, the nerve supply of the auricle, the nerve supply of the palate, the nerves to the submaxillary and sublingual glands, and the nerves in the tongue.

I am greatly indebted to Prof. A. G. Pohlman for permission to use his graphic method in advance of its publication, to Prof. Burton D. Myers for library facilities extended to me on numerous occasions, and to both of them for helpful suggestions during the course of this work.

MOTOR PART OF THE NERVUS FACIALIS

The nucleus of origin and the central course and relations of the motor part of the nervus facialis of the mouse are very similar to those of other mammals and man. The nucleus is located in the ventral part of the pons close to its surface, and just medial and slightly ventral to the nucleus olivaris superior. This superficial position is due to the absence of transversely directed pontine fibers other than those of the trapezoid body.

The nucleus is composed of large multipolar cells closely resembling in size and shape those in the oculomotor and hypoglossal nuclei. Coarse fibers arise from the cells of the nucleus and pass dorsally with a slight inclination medially, scattered over a considerable area of the formatio reticularis. In relation to the dorsal surface of the nucleus abducens the fibers unite into a compact bundle. This bundle passes anteriorly for a short distance and bends at an angle forming the genu internum.

The emerging part of the nerve passes ventrally, laterally, and slightly anteriorly until near the ventral surface of the pons. Here it makes a bend and emerges from the pons by passing almost horizontaly laterally through the fibers from the cochlear nuclei and between the nucleus and radix spinalis nervi trigemini on its dorsal side, and the anterior extremity of the nucleus olivaris superior on its ventral side.

After emerging from the pons the facial nerve passes laterally, dorsal to the cochlea and the tensor tympani muscle, to the dorsal wall of the tympanic cavity where it bends posteriorly in the genu externum. At the surface of the pons the nerve is in relation to the emerging trigeminal nerve anteriorly, the vestibular nerve and ganglion dorsally, and the cochlear nerve posteriorly (fig. 1). The relation to the vestibular ganglion at this place is accounted for by the fact that it is located against the surface of the pons, anterior and inferior to the ventral cochlear nucleus, and overlapping a part of it.

Dorsal to the facial nerve between the pons and the genu externum, are the vestibular nerve and ganglion, the nervus intermedius, and the ramus ampullae superior and ramus ampullae

lateralis of the vestibular nerve. Anterior to it close to the pons, is the trigeminal nerve. More laterally a thin shell of bone and the geniculate ganglion separate it from the cranial cavity (figs. 2, 3, and 9). Posteriorly the facial nerve is overlapped by the vestibular ganglion and is related, farther laterally, to the ampullae of the semicircular canals.

The genu externum lies between the ampullae of the semicircular canals and the stapedial artery. The bend is a little more than a right angle. In the dorsal wall of the tympanic cavity the facial nerve lies dorsal to the stapedial artery and the stapes, and lateral to the stapedius muscle. Behind the tympanic cavity it bends ventrally and anteriorly with an inclination laterally, so that as the nerve passes anteriorly it lies ventral to the external auditory meatus.

From this position the facial nerve passes forward just lateral to the attachment of the cartilage of the auricle to the bone and beneath the parotid gland. Anterior to the meatus it gives off its cervical branches and divides into four parts.

The branches and distribution of the motor part of the facial nerve can be briefly summarized as follows (fig. 14 A):

1. Cervical branches. Four or five small branches arising from the trunk of the nerve and passing ventrally and posteriorly, some between the lobules of the parotid gland, some beneath it, to supply the superficial muscle (panniculus carnosus, musculus cutaneus) of the neck and the region superficial to the parotid gland.

2. Temporopalpebral branches. Branches arising immediately anterior to the preceding and passing anteriorly and dorsally beneath the parotid gland. These are accompanied by the auriculotemporal nerve and are distributed to the muscles anterior to the auricle. One small palpebral branch continues farther anteriorly and ends in the muscles of the upper eyelid.

3. Buccal branches. Two large branches which compose the greater part of the nerve, the upper being accompanied by a few fibers from the auriculotemporal nerve. These pass in a horizontal direction across the cheek, beneath the parotid gland at first, then beneath the cutaneous muscle. Their fibers supply

the muscles around the angle of the mouth, and those of the lower eyelid, nose, and upper lip, including the muscles of the vibrissae.

4. Inferior labial branch. A small branch which passes ventral to the angle of the mouth to supply the muscles of the lower lip.

The anatomical and clinical evidence for the motor function of these fibers is so conclusive that they were not followed as closely as were the other branches of the facial nerve. The cervical,

ABBREVIATIONS

Aur-Pal.Brs., auriculopalpebral branches of the nervus facialis
Buc.Brs., buccal branches of the nervus facialis
C.C., cranial cavity
Cer.Brs., cervical branches of the nervus facialis
G.Gen., ganglion geniculi
G.Gen.d., dorsal part of the ganglion geniculi
G.Ot., ganglion oticum
G.Sem., ganglion semilunare
G.Sp., ganglion sphenopalatinum
G.Vest., ganglion vestibulare
H.Can.Fac., hiatus canalis facialis
Inf.Lab.Brs., inferior labial branches of the nervus facialis
M., mouth cavity
M.Ten.Tym., musculus tensoris tympani
M.St., musculus stapedius
N., nasal cavity
N.Abd., nervus abducens
N.Can.Pt., nervus canalis pterygoidei
N.Ch.Ty., nervus chorda tympani
N.Coch., nervus cochlearis
N. to Dia., nerve to musculus digastricus
N.Fac., nervus facialis
N.Int.e., efferent part of the nervus intermedius
N.Int.l., lateral part of the nervus intermedius
N.Int.m., medial part of the nervus intermedius
N.Ling., nervus lingualis
N.Man., nervus mandibularis
N.Max., nervus maxillaris
N.Np., nervus nasopalatinus
N.Oc., nervus oculomotorius
N.Oph., nervus ophthalmicus
N.Pal.A., nervus palatinus anterior
N.Pal.M., nervus palatinus medius
N.Pal.P., nervus palatinus posterior
N.Sp., nervi sphenopalatini
N.Stap., nervus stapedius
N. to St-Hy., nerve to musculus stylohyoideus
N.P.P., nervus petrosus profundus
N.P.S.M., nervus petrosus superficialis major
N.Tr., nervus trochlearis
N.Tri., nervus trigeminus
N. to tym.memb., nerve to tympanic membrane and external auditory meatus
Orb.Brs., orbital branches of the ganglion sphenopalatinum
P.Inf.Na., posterior inferior nasal nerve
R.Aur.N.V., ramus auricularis vagi
R.Aur.P., nervus auricularis posterior
R.Cut.N.F., ramus cutaneus facialis
Sym., sympathetic plexus along the internal carotid artery
T.C., tympanic cavity

auricular, and palpebral branches were followed to their termination in the muscles. These pass beneath the cutaneous muscle, while the sensory nerves for the supply of the skin of the same regions are superficial to the muscle, an arrangement which makes it possible for the motor nerves to be easily followed.

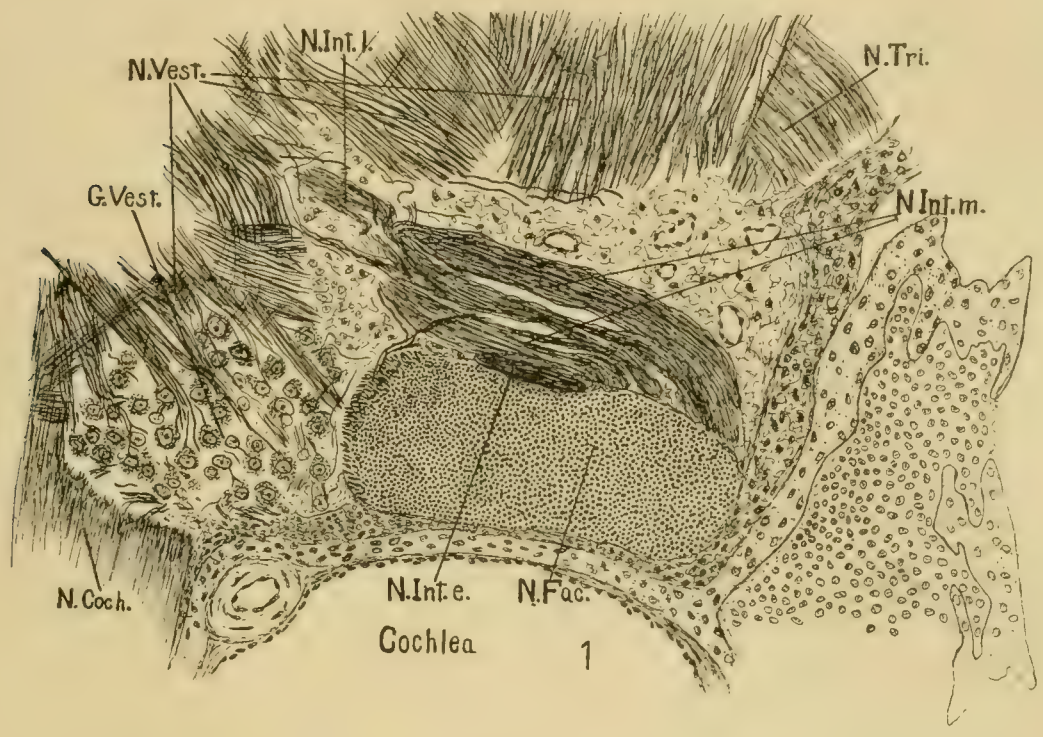


Fig. 1 Sagittal section through the nervus facialis and the nervus intermedius close to the surface of the pons. Figures 1, 2, 3, and 9 are from the same series. × 166.

In the upper lip and in the eyelids the branches of the facial nerve are intermingled with those of the trigeminal nerve. This is particularly true around the roots of the vibrissae in the upper lip where the trigeminal fibers are exceedingly numerous (Vincent, '13). Some of the bundles of facial fibers were followed through this plexiform mass in each of these regions and were found to terminate in muscular tissue.

SENSORY PART OF THE NERVUS FACIALIS

1. Ganglion geniculi

The geniculate ganglion in the mouse is divided into two portions continuous with each other anterior to the facial nerve. The larger, the main part of the ganglion (fig. 3, *G.Gen.*) is located half way between the superficial origin of the facial nerve and the genu externum. It is elongated triangular in shape, the long axis extending anteriorly and ventrally. The anterodorsal surface is separated from the cerebrum by the cranial wall, the tip of the ganglion extending through the hiatus canalis facialis as far as the posterior extremity of the semilunar ganglion. The postero-ventral surface is separated by the bone from the cochlea posteriorly and the tensor tympani muscle anteriorly. The posterodorsal border of the ganglion is in relation to the anterior border of the motor part of the facial nerve.

The fibers of the nervus intermedius leave the ganglion at its medial angle, those fibers of the nervus intermedius which pass peripherally in the facial nerve emerge at the lateral angle, and from its anterior angle the great superficial petrosal nerve takes origin.

The above description corresponds closely to that of Penzo ('93) and Weigner ('05) except that the ganglion is not located at the genu, but medial to it.

The dorsal and smaller part of the geniculate ganglion (figs. 2 and 3, *G.Gen.d.*) is placed dorsal and medial to the main portion. It is located in a triangular interval between the motor part of the facial nerve ventrally, the vestibular ganglion dorsally and the cranial wall anteriorly. It is separated by a thin layer of connective tissue from the vestibular ganglion and is intimately related to the nervus intermedius. It is possible that this part of the ganglion in the mouse represents the numerous scattered ganglion cells along the nervus intermedius described by Weigner ('05) in the ground-squirrel.

The cells of the geniculate ganglion are approximately half the size of those of the semilunar and dorsal root ganglia. They are mostly of uniform size with only a few larger and smaller

cells scattered among the others. They are of the unipolar type in which the single process is convoluted and twisted around the cell body (Ranson, '12, type 1). No collaterals ending in end-bulbs were seen, although this type is particularly numerous in sections of the human geniculate ganglion in my possession. It was at first thought that the absence of these might be due to the fact that they had not developed. Huber and Guild ('13 b) found them in the spinal ganglia of three-day-old rats and they are present in the semilunar and spinal ganglia of my mice. It is probable, therefore, that they are few in number or absent in the geniculate ganglion of the mouse.

That the geniculate ganglion belongs to the cerebrospinal type of ganglia and is composed of unipolar cells was first established by Retzius ('80) and has since been confirmed by von Lenhossék ('94) and Weigner ('05).

2. Nervus intermedius

Between the geniculate ganglion and the brain the nervus intermedius is composed of two parts, the larger contains those fibers which come from the interior of the geniculate ganglion, the smaller being a separate efferent root of the nerve (fig. 1).

The fibers from the ganglion leave it in two distinct bundles. The more laterally placed and smaller bundle (figs. 1, 2, and 3, *N.Int.l.*) leaves the upper border of the main part of the ganglion and passes dorsally and medially anterior to the dorsal part of the ganglion. At the dorsal limit of this part of the ganglion this bundle makes an abrupt bend posteriorly and extends through the vestibular ganglion (fig. 2). Leaving this ganglion it passes on to the dorsal surface of the motor part of the facial nerve and is joined by the other bundle (fig. 1).

There is apparently some mixing of fibers during the passage of this bundle through the vestibular ganglion. Whether there is an actual interchange of fibers in the form of anastomoses could not be determined. It is certain, however, that there are no large contributions from one to the other, for the difference in the size of the fibers would make possible the detection of such.

The medial and larger bundle of fibers from the geniculate ganglion takes a more direct course into the pons (figs. 1, 2, and 3, *N.Int.m.*). Arising from the medial angle of the ganglion it passes obliquely posteriorly and medially across the dorsal surface of the motor part of the facial nerve. At the surface of the pons it is joined by the lateral bundle, the two having a common central course.

The vestibular ganglion lies dorsal to and in intimate contact with this bundle. In some sections bipolar ganglion cells were

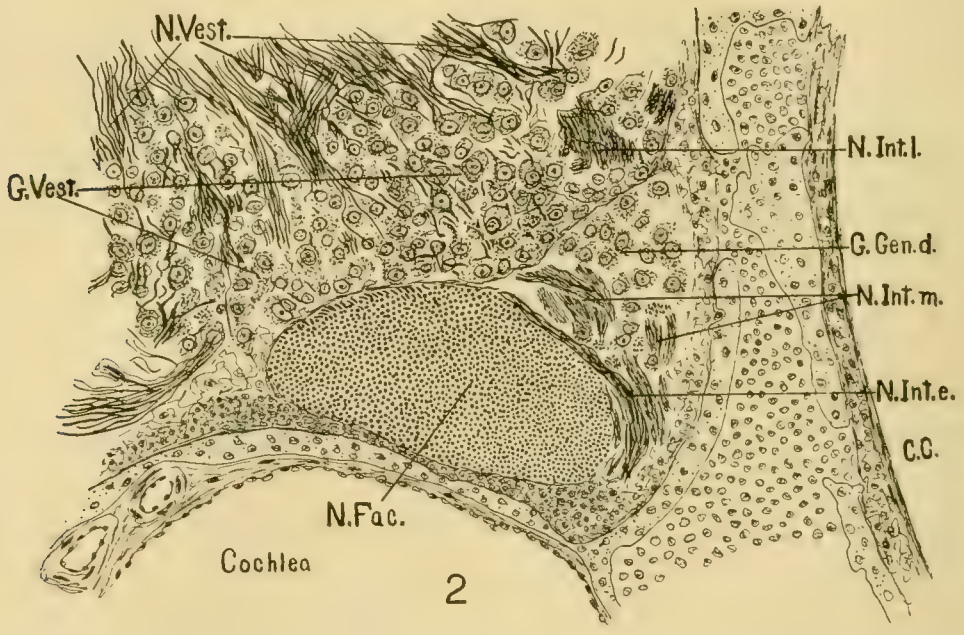


Fig. 2 Sagittal section through the nervus facialis, the nervus intermedius, and the dorsal part of the ganglion geniculi. 75 μ lateral to figure 1. $\times$ 166.

found along it. Other than these there are no evidences of anastomoses between the nervus intermedius and the vestibular nerve.

The nervus intermedius, in cross sections through the place of union of its two bundles, is circular in outline. It is located in a triangular interval bounded ventrally by the emerging motor fibers, dorsally by the nervus and ganglion vestibulare, and meially by the surface of the pons. From this position it can be followed into the interior of the pons. Here it passes dorsally, posteriorly and medially through the fibers from the cochlear

nuclei and the spinal root of the trigeminal nerve into the nucleus of the latter. In this the fibers become scattered, and because of the intricate complex of fibers running in all directions at this point, they cannot be followed farther.

The efferent root of the nervus intermedius is most conspicuous in sagittal sections where it, too, has an oblique course across the surface of the motor part of the facial nerve (figs. 1 and 2

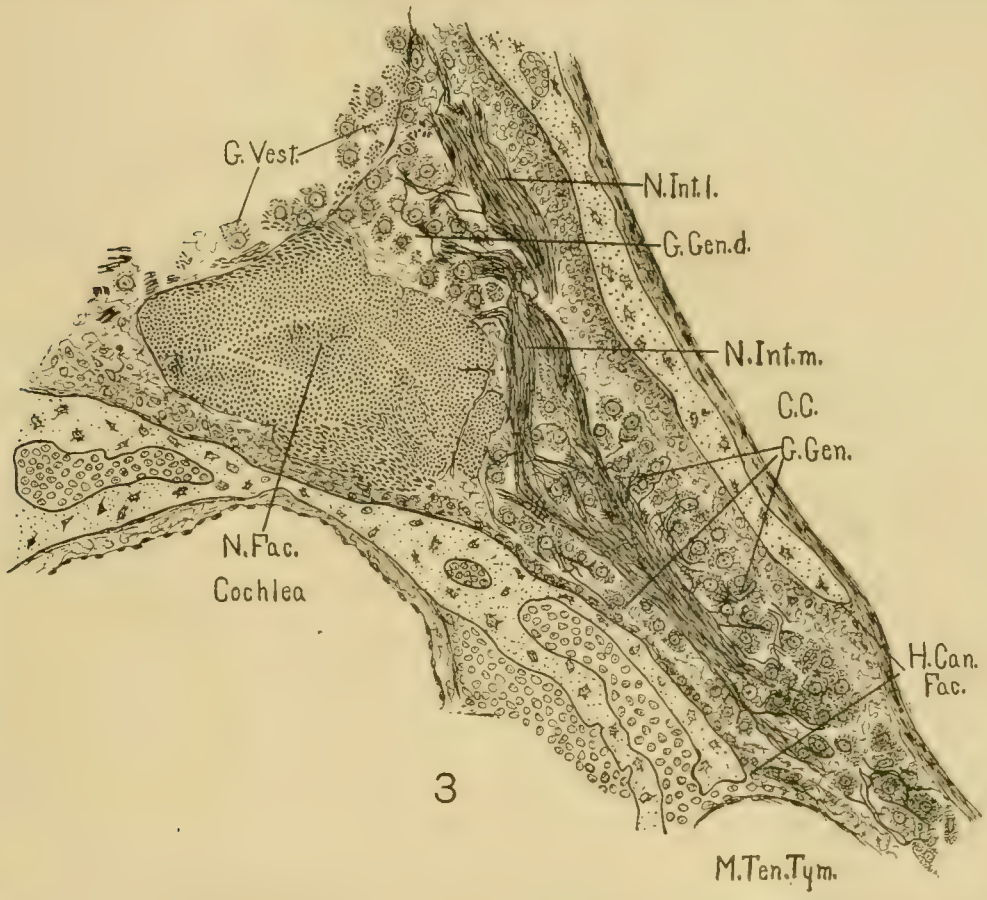


Fig. 3 Sagittal section through the nervus facialis and the ganglion geniculi. 60 μ lateral to figure 2. $\times$ 166.

N.Int.e.). Followed centrally from this position, it passes posteriorly and medially for a short distance across the surface of the pons. At the ventral edge of the spinal root of the trigeminal nerve it enters the pons and passes dorsally and medially, parallel with, but more lateral and posterior to the emerging motor part of the facial nerve. At the level of the ascending part of the facial nerve the compact bundle becomes broken up and the fibers scattered, so that they cannot be followed farther.

Peripherally, the efferent root of the nervus intermedius passes around the anterior border of the motor part of the facial nerve, under the rest of the nervus intermedius, through the medial border of the geniculate ganglion and into the great superficial petrosal nerve. It is certain that only a few, if any, of its fibers are connected with the cells of the geniculate ganglion. This part of the nervus intermedius is present in all my series and undoubtedly contributes efferent fibers to the great superficial petrosal nerve.

In the material used in this work it was impossible to tell what proportion of the fibers of the nervus intermedius were medullated and what non-medullated. All of the axis-cylinders are small in diameter and the medullated sheaths must be very thin. Weigner ('05) has given an excellent description of the fibers of the nervus intermedius of the ground-squirrel. In teased preparations stained with osmic acid he found that most of them are very fine with very delicate myelin sheaths (2 μ in diameter), that a few are larger (5 μ), and that the nerve contains nonmedullated fibers. The fibers of the nervus intermedius of the mouse are probably similar in size and character.

Weigner describes numerous anastomoses between the nervus intermedius, the vestibular nerve, and the facial nerve, which are characterized by the presence of ganglion cells, scattered and in groups, along the anastomosing bundles. These ganglion cells, he states, are similar to those of the geniculate ganglion and furnish additional centers of origin for nervus intermedius fibers. In the mouse, no ganglion cells are present central to the dorsal part of the ganglion and no true anastomoses were observed.

That the afferent fibers of the nervus intermedius terminate in the anterior extremity of the nucleus of the fasciculus solitarius or a group of cells lying anterior to it seems well established. All of the available papers dealing with this point in lower vertebrates state this to be true. Van Gehuchten ('00) found this to be the termination of these fibers in rabbits. The efferent fibers to the submaxillary and sublingual glands arise from a nucleus in the formatio reticularis at the level of the motor facial nucleus. This nucleus was called the nucleus salivatorius

by Kohnstam ('02) and has been described by Yagita and Hayama ('09). Herrick ('16) calls it the nucleus salivatorius superior. The efferent fibers of the great superficial petrosal nerve arise from other cells in the same region (Yagita, '14).

In the mouse, three distinct bundles of nerve fibers continue the nervus intermedius peripheral to the ganglion. From the anterior angle of the ganglion the great superficial petrosal nerve emerges, while from its lateral angle two bundles of fibers pass peripherally in a common sheath with the motor fibers of the facial nerve. Before the genu is reached these bundles lie along the antero-ventral side of the facial nerve, the smaller of the two lying ventral to the larger (figs. 9 and 10, *N.Ch.Ty.*, *R.Cut.N.F.*).

In sagittal series medial to the genu these bundles are always more or less separated by delicate connective tissue septa from each other and from the motor fibers of the facial nerve. Beyond the genu they lie along the lateral side of the nerve (fig. 10). In this position the septum is not as distinct as it is nearer the ganglion. They are well separated in some series, while in others an indentation along their medial side is all that indicates a division into two parts. The ventral and smaller of these two bundles becomes the chorda tympani, the dorsal and larger contains fibers which are distributed to the skin of the auricle and form a nerve which I have called the ramus cutaneus facialis.

With the exception of the efferent fibers of the great superficial petrosal nerve, it was impossible in this material to tell the exact relation of the fibers of the nervus intermedius to the cells of the geniculate ganglion. That some of the fibers are connected with the cells of the ganglion and others pass through without interruption seems well established.

Cutting the chorda tympani in the middle ear results in a degeneration of about four-fifths of the cells of the geniculate ganglion (Amabilino, '98; DeGaetani, '06). Nissl degeneration of nerve cells in the brain stem after cutting the fibers to the submaxillary and sublingual glands is proof that the chorda tympani contains fibers which do not have their fibers in the ganglion (Kohnstam, '02; Yagita and Hayama, '09).

There is also considerable evidence for the presence of both afferent and efferent fibers in the great superficial petrosal nerve. By staining the geniculate ganglion of the mouse by the Golgi method von Lenhossék ('94) found fibers which pass directly from the nervus intermedius into the great superficial petrosal nerve. From this he erroneously concluded that the great superficial petrosal is a motor nerve for the supply of the levator veli palatini and levator uvulae muscles. After cutting the great superficial petrosal nerve in dogs, Yagita ('14) found that about one-twelfth of the cells of the geniculate ganglion show typical Nissl degeneration, and that there were degenerated cells in the formatio reticularis of the same side of the pons extending from the middle to the upper third of the facial nucleus.

Weigner ('05) mentions a bundle which passes directly from the facial nerve to the great superficial petrosal through the human geniculate ganglion. In the ground-squirrel he describes a bundle between the nervus intermedius and the great superficial petrosal nerve which has no connection with the ganglion cells. He could not determine, he states, whether the fibers of this bundle are processes of the scattered cells in the nervus intermedius or of those in the great superficial petrosal nerve.

It is safe to conclude, therefore, that both the chorda tympani and the great superficial petrosal nerve contain afferent fibers whose cell bodies are located in the geniculate ganglion, and efferent fibers which pass through the ganglion without connection with its cells.

BRANCHES OF THE NERVUS FACIALIS

1. Nervus petrosus superficialis major

It has been shown above that the nervus petrosus superficialis major arises within the cranial cavity from the anterior pointed extremity of the ganglion geniculi, and that it is composed of fibers whose cell bodies are located in the ganglion, and others which form a separate efferent root of the nervus intermedius.

From its origin this nerve passes anteriorly for a short distance along the lateral side of the ventral surface of the ganglion semi-

lunare. After a short course it bends at almost a right angle and passes medially, ventral to the nervus trigeminus, and ventral to the internal carotid artery and the sympathetic plexus which surrounds it. At a point just medial to the artery the nerve bends anteriorly and is joined along its medial side by the nervus petrosus profundus, the two uniting to form the nervus canalis pterygoidei.

A. Nervus petrosus profundus. The nervus petrosus profundus, in the mouse, is formed by two small bundles of fibers from the internal carotid plexus. It does not have a separate course for the fibers join the great superficial petrosal nerve immediately after leaving the plexus and while still in relation to the artery.

The fibers composing this nerve can readily be followed to the interior of the superior cervical sympathetic ganglion. Along the nerve of the pterygoid canal they occupy, at first, a position on its medial side Farther anteriorly, however, they become intermingled to such an extent with those from the great superficial petrosal nerve that the fibers from the two sources cannot be separately identified.

B. Nervus canalis pterygoidei. In mice, the internal carotid artery enters the cranium and the nerve of the pterygoid canal leaves it through a slit-like fissure between the tympanic and periotic bones (eustachian aperture). From this fissure the nerve passes anteriorly and slightly medially along the ventral surface of the body of the sphenoid bone. One of the pharyngeal muscles (probably the salpingo-pharyngeus), the pharyngeal opening of the auditory tube and a mass of glands lie ventral to it. At the anterior border of the opening of the auditory tube the muscle and glands disappear and the nerve lies between the bone and the mucous membrane of the nasal cavity. At this place the pterygoid process extends ventrally to the soft palate, the nerve lying medial to it where it fuses with the body of the bone above. In this position the nerve extends anteriorly for some distance finally passing laterally through a foramen into the most posterior part of the orbit.

The nerve of the pterygoid canal is, at first, flattened dorso-ventrally becoming circular in outline more anteriorly with the

fibers more loosely arranged. All of its fibers are very fine. There are no ganglion cells either along the great superficial or the deep petrosal nerves and only a few along the nerve of the pterygoid canal. With the exception of one elongated microscopic ganglion about the middle of its course, these are single and widely separated.

In one series a few fibers were given off from the nerve of the pterygoid canal to the mass of glands lying dorsal to the auditory tube. Similar fibers could not be found in any other series, nor were there other branches from this nerve.

In addition to the sympathetic fibers to the sphenopalatine ganglion by way of the nervus petrosus profundus and the nervus canalis pterygoidei, others from the internal carotid plexus reach the ganglion by way of the nervus abducens. These fibers join the nervus abducens as two bundles slightly anterior to the point of union of the two petrosal nerves. They leave the nervus abducens as four small bundles, two of which join the ophthalmic nerve and the other two the sphenopalatine ganglion just posterior to its middle (figs. 4 and 7, bundles *b* and *c*).

Koch ('16) mentions the presence of sympathetic nonmedullated fibers from the cavernous plexus in the nervus abducens of the dog. These leave the nerve more anteriorly to pursue an independent course. Piersol ('13) states that branches of the sphenopalatine ganglion have been described joining the nervus abducens.

C. Ganglion sphenopalatinum. It will be shown later that none of the fibers of the sphenopalatine nerves end in the sphenopalatine ganglion. This ganglion belongs, therefore, more to the facial than to the trigeminal nerve. Most of the fibers from it, however, are distributed as constituents of the palatine nerves. For this reason, it has been found necessary to study the ganglion itself and the nerves connected with it in any way.

The sphenopalatine ganglion in the mouse is an elongated mass of ganglion cells lying between the medial side of the maxillary nerve and the medial wall of the orbit. It begins as a small accumulation of cells along the ventral border of the nerve of the pterygoid canal immediately after that nerve enters the

orbit. Anteriorly, the ganglion gradually increases in size, the cells completely surrounding the nerve. This continues until the anterior limit is reached where it ends in a blunt extremity.

The posterior half of the ganglion is flattened laterally and is, in cross-section, an elongated oval with the long axis extending



Fig. 4 Transverse section through the most posterior part of the orbit showing the nervus oculomotorius, nervus trochlearis, nervus ophthalmicus, nervus maxillaris, nervus abducens, the posterior part of the ganglion sphenopalatinum and the origin of the nervus palatinus posterior. By referring to figures 7 and 8 the connections of the nerve bundles marked with small letters and arabic numerals will be seen. Figures 4, 5, and 6 are from the same series. × 90.

vertically (fig. 5). Near its middle the ganglion changes its shape and becomes flattened dorsoventrally (fig. 6). At this point a ventral bend in the ganglion together with its change in shape imperfectly separates it into a posterior and an anterior part.

Medially the sphenopalatine ganglion is separated by the bone from the nasal cavity. Laterally it is related to the maxillary nerve throughout its entire extent, the sphenopalatine nerves extending ventrally along its lateral side at about its middle.

The sphenopalatine ganglion of mammals is described as receiving the sphenopalatine nerves from the maxillary nerve and as giving off branches which are distributed to the mucous membrane of the nose, mouth and pharynx, and branches to the orbit. All of the nerves which are usually mentioned in connection with the ganglion, with the exception of the pharyngeal branch, are represented in the mouse by similar nerves.

a. Nervi palatinus posterior et medius. The posterior and middle palatine nerves arise from the lateral side of the maxillary nerve widely separated and distinct from both the sphenopalatine ganglion and the sphenopalatine nerves. They will be described, therefore, before the sphenopalatine nerves.

The posterior palatine nerve is represented by one or two small nerves coming from the lateral side of the maxillary nerve at the level of the middle of the posterior part of the sphenopalatine ganglion (fig. 4, *N.Pal.P.*). It is separated by the maxillary nerve from the sphenopalatine ganglion, and arises from a part of the maxillary nerve which gives off branches for the supply of the teeth and gums of the upper jaw.

The posterior palatine nerve receives two small bundles of fine fibers from the ventral border of the sphenopalatine ganglion (fig. 4, *d,e*). These pass laterally, ventral to the maxillary nerve, and join the posterior palatine immediately before it enters the canal in the bone through which it passes to the palate. They are so small that in some series it is difficult to follow them even by using the high power of the microscope. Unsuccessful attempts were made to count the fibers they contain. I believe it safe to conclude, however, that they do not exceed thirty-five or forty in number.

The middle palatine nerve is represented by a single smaller branch which takes origin from the ventral side of the maxillary nerve. This nerve passes inferiorly where it, too, enters a canal

in the bone and extends to the mucous membrane of the palate. In some series it was possible to follow a minute bundle from the sphenopalatine ganglion into it; in other series this could not be done. When present this bundle is even smaller than the ones joining the posterior palatine.

The termination of these nerves will be discussed later.

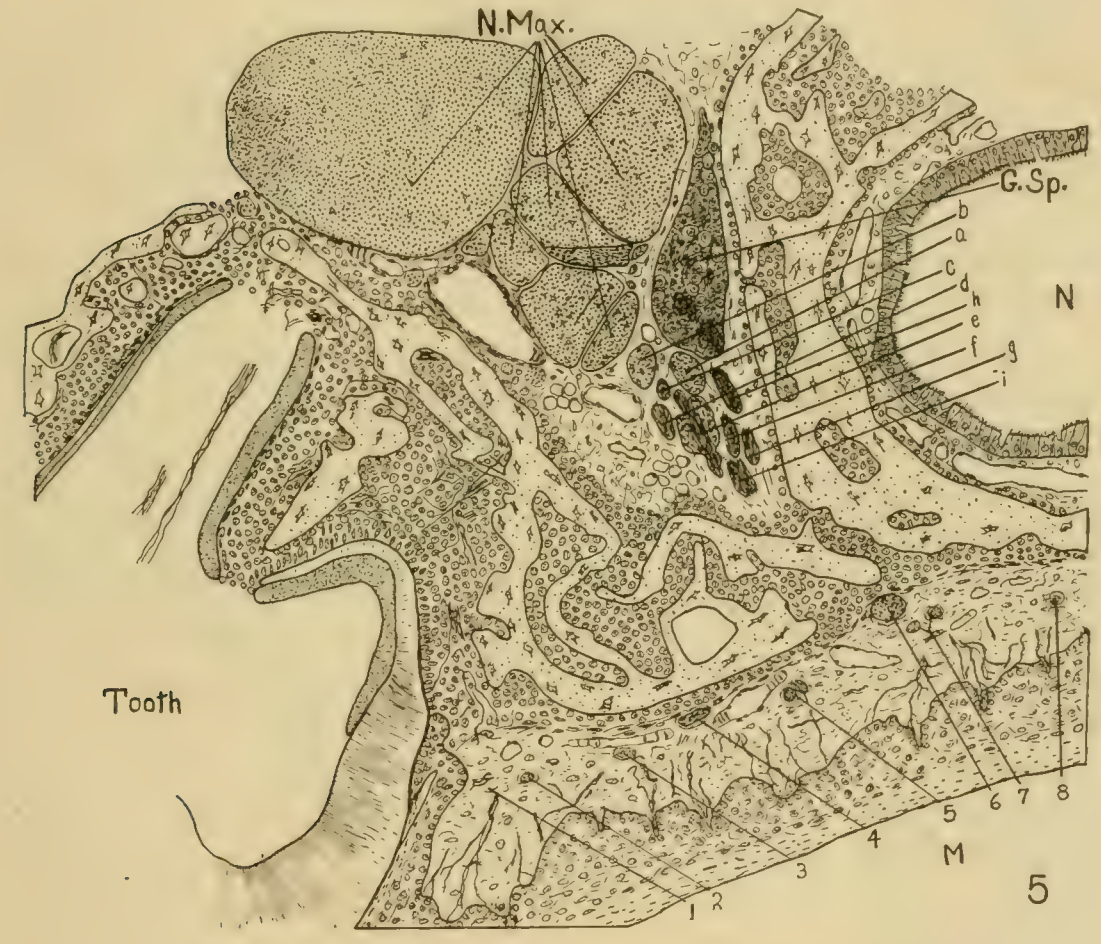


Fig. 5 Transverse section through the nervus maxillaris, the ganglion sphenopalatinum, and the nervi sphenopalatini. 810 μ anterior to figure 4. $\times$ 90.

b. Nervi sphenopalatini. The sphenopalatine nerves take origin from the ventromedial part of the maxillary nerve at about the middle of the sphenopalatine ganglion (fig. 5, bundles *a*, *b*, *c*, *d*, *e*, *g*, *i*, and part of *f*.). The bundles of fibers present in these nerves vary in different series. In the one represented in the graph, figure 7, and from which figures 4, 5, and 6 were drawn, there were six distinct bundles at their point of origin.

Almost immediately their arrangement becomes so complicated that it cannot be easily described. This arrangement is, however, accurately represented in the graph (fig. 7, *N.Sp.*). A greater part of five of the six bundles assist in forming the anterior palatine nerve and its posterior inferior nasal branch, and the greater part of the other bundle assists in forming the nasopalatine nerve.

From these six bundles there are only two small subdivisions that enter the sphenopalatine ganglion, joining it well toward its anterior end and close to the point of origin from the ganglion of the fibers which enter the nasopalatine nerve. In transverse series these bundles can be followed through the ganglion into the nasopalatine nerve, which is probably their fate in the other series.

c. Nervus palatinus anterior. The anterior palatine nerve is the largest of the nerves in this region. The bundles from the maxillary nerve which form it pass medially and anteriorly across the ventral border of the sphenopalatine ganglion. While in this position they are joined by four bundles whose fibers come from the interior of the sphenopalatine ganglion. The fibers from the ganglion are smaller than those from the maxillary nerve and represent about one-third of the fibers of the anterior palatine nerve.

From the ventral border of the sphenopalatine ganglion the anterior palatine nerve passes ventrally through a canal to reach the mucous membrane of the palate. In this canal one bundle separates from the nerve, and as soon as the mucosa is reached, takes a posterior direction. The remainder of the nerve passes anteriorly just lateral to the middle of the hard palate.

The posterior inferior nasal branch of the anterior palatine nerve is made up of two small bundles of fibers from the sphenopalatine nerves (fig. 6, *a*, *b*) and four smaller bundles of fine fiber from the sphenopalatine ganglion (fig. 7). This nerve extends anteriorly along the lateral wall of the nasal cavity, the smaller fibers soon leaving it to enter a mass of glands in the lateral wall of the nose (gland of Stenson). The fibers of larger size end in the mucous membrane of the lateral wall of the nasal cavity.

d. Nervus nasopalatinus. One bundle of the sphenopalatine nerves passes obliquely forward and medially across the inferior surface of the anterior part of the sphenopalatine ganglion, finally reaching the medial border of the anterior end of the ganglion (fig. 5, bundle *a*). In this position it receives a large contribution of fine fibers from the ganglion and becomes the nasopala-

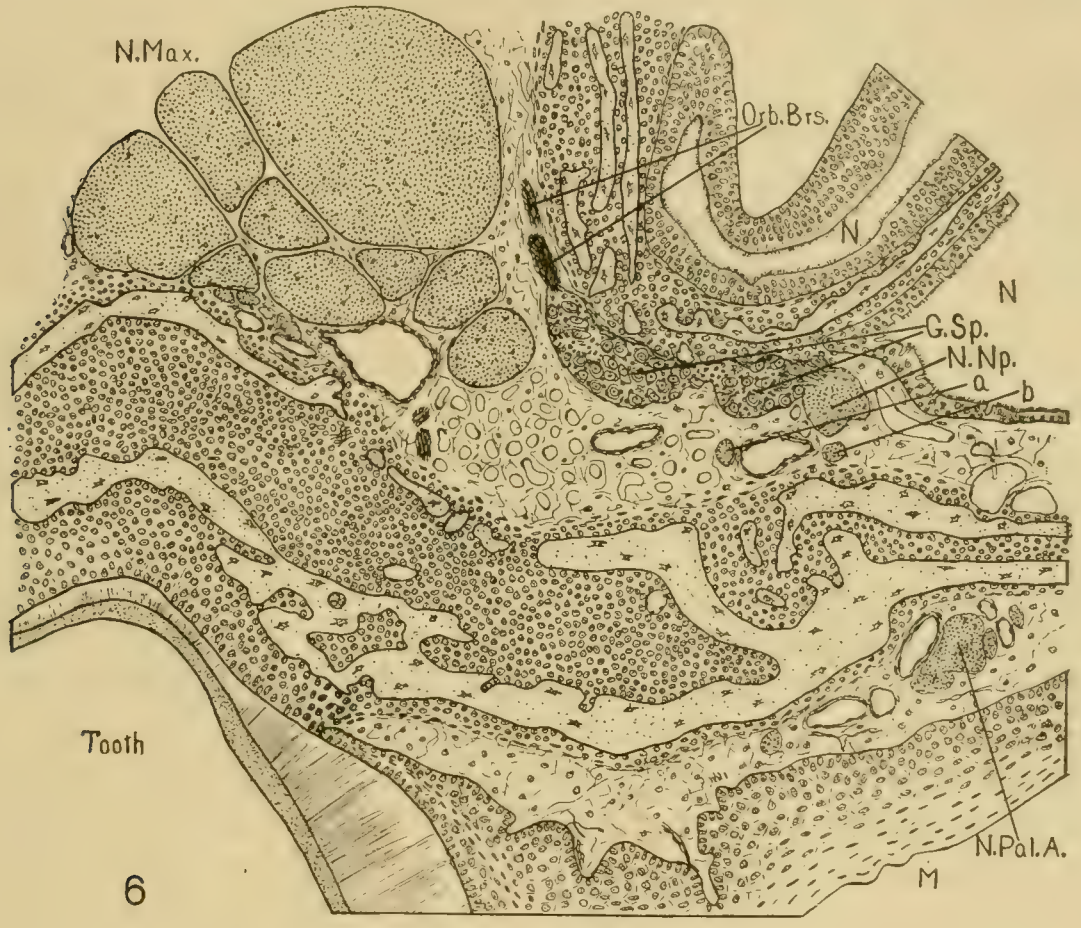


Fig. 6 Transverse section through the nervus maxillaris and the anterior extremity of the ganglion sphenopalatinum showing the origin of the nervus nasopalatinus and two of the orbital branches of the ganglion. 600 μ anterior to figure 5. $\times$ 90.

tine nerve (fig. 6). The fibers from the ganglion form a little more than half of this nerve.

From its origin the nasopalatine nerve extends medially along the posterior wall of the inferior meatus of the nose to reach the posterior and ventral part of the septum. From this point its course is anterior along the ventral part of the septum. Through-

out its extent branches are given off, some of coarse fibers to the mucous membrane, and others composed of finer fibers from the sphenopalatine ganglion which pass into the glands of the septum. The septal glands are numerous in the region posterior to the vomeronasal organ. Finally, reduced in size and composed only of fibers of larger size, the nasopalatine nerve passes through the incisive foramen to reach the roof of the mouth.

e. Other branches. In addition to the fibers from the sphenopalatine ganglion described above, there are several other small nerves either arising or ending within it. One small nerve extends from the ganglion posteriorly along the medial side of the semilunar ganglion (fig. 7). Several other small bundles extend from the sphenopalatine ganglion dorsally and laterally to join the ophthalmic nerve. (These are not shown in the graph, fig. 7.) The origin, the direction or the endings of these nerves could not be determined.

Several small nerves take origin from the anterior part of the sphenopalatine ganglion and pass dorsally along the medial wall of the orbit. Some of these join the nasociliary nerve, and through it finally terminate among the gland ducts of the anterior and inferior part of the lateral wall of the nasal cavity. Others accompany a blood-vessel into the medial part of the lacrimal gland, while one other joins a small branch of the maxillary nerve which terminates in the nasolacrimal duct.

Other branches from the anterior end of the ganglion join the arteries in the neighborhood and accompany them in their peripheral courses.

f. The nerve supply of the palate. The nerve supply of the palate was investigated to determine, if possible, the relationship between the nerves of the taste-buds, the nerves to the palatal glands, and the facial nerve. Although no positive conclusions were reached, certain features are of interest and will be recorded here.

From the ventral end of its bony canal the anterior palatine nerve extends anteriorly, in company with a medium-sized artery, as far forward as the nasopalatine canal. Throughout its entire extent it sends branches medially and laterally to supply

one-half of the palate. Anterior to the nasopalatine canal the mucous membrane of the medial part of the palate is supplied by the terminal branches of the nasopalatine nerve, that of the lateral part by a branch of the maxillary nerve.

Branches from these nerves are abundant under the transverse ridges of the palate and particularly so around the nasopalatine canal. In the tunica propria the nerve fibers form rather wide meshed plexuses from which individual fibers can be followed into the epithelium. No encapsulated nerve endings were found. In this area of the palate there are no taste-buds or glands. From the anterior palatine nerve many fine fibers can be followed along the walls of the blood-vessels, presumably to end in their muscle fibers, although this termination could not be positively identified.

The graph (fig. 8) shows the distribution of the middle and posterior palatine nerves and the posteriorly directed branch of the anterior palatine nerve to the posterior part of the hard palate and the soft palate. The transverse line *X* shows the place of union of the hard and soft palate. The graph includes one-half of the palate, the right margin representing the median line and the left margin the lateral edge. In this connection it must be remembered that the anterior part of the palate shown in the upper part of the graph is wider than the posterior part, thus accounting for the apparent preponderance of nerves in the upper part of the graph. The small circles show the approximate locations of the taste-buds, and the broken line represents the limits of the palatal glands.

In the series represented in the graph, there were twenty-five taste-buds, twelve in the posterior part of the hard palate, and thirteen in the soft palate. In other series the number is approximately the same and the arrangement is similar. They are most numerous along the posterior part of the hard palate and gradually decrease in number posteriorly.

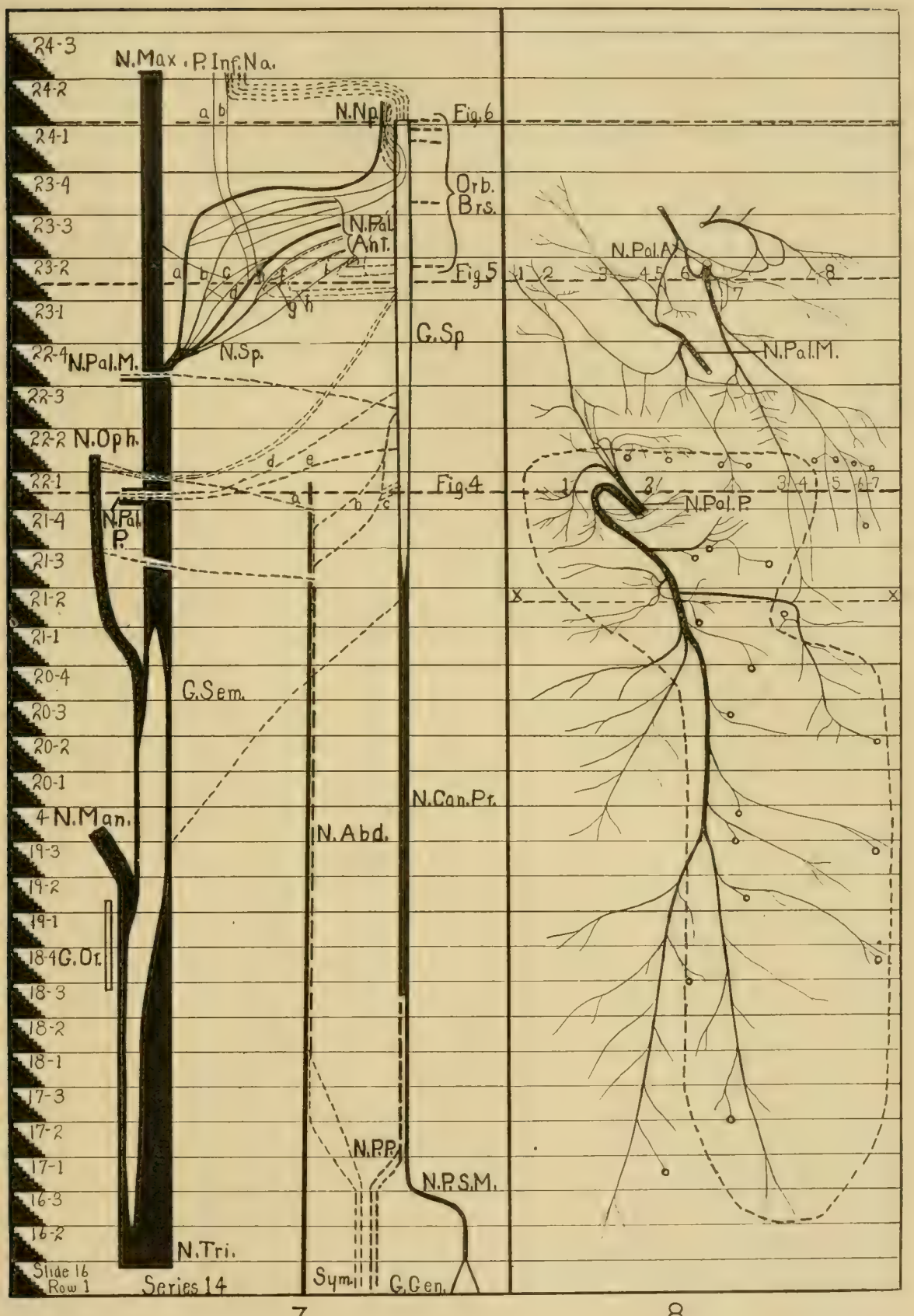
The nerves of this region can be followed from their emergence from the bony foramina to their termination. Those supplying the epithelium can be traced to the basement membrane and some of the fibers were seen to pass among the epithelial cells.

Fig. 7 A graph, made according to the method devised by Prof. A. G. Pohlman, showing the connections of the nervus petrosus superficialis major and the ganglion sphenopalatinum. A graph of this sort is made by selecting that part of a series which is to be used, and marking out on one edge of a piece of millimeter plotting paper a set of stair steps for each row of sections on the slides, representing each section by a single step. Nerves or other structures are represented in the graph by broken or solid lines, dots, etc., and as these structures continue through the series the lines or dots are continued on the paper, passing through a millimeter for each section. By continuing this and representing changes in position, branchings, anastomoses, etc., by changes in the lines, almost any structure which passes for some distance through a series can be graphically shown.

The graph shown as figure 7 represents certain nerves and ganglia and their connections in slides 16 to 24 of a transverse series of one half of a mouse head. The numbers on the left of the graph are the numbers of the slides and the rows on each slide. Each section is represented by a single step in the stairs. As an illustration, the ganglion geniculi is present in section 1, row 1, slide 16, and is shown at the lower right part of the graph. The ganglion ends and the nervus petrosus superficialis major begins in the last section in that row. This nerve continues through the second into the third row where it bends medially, this being shown by a bend in the line representing the nerve. In section 8, row 1, slide 17, and in section 2 of the second row the nervus petrosus profundus joins the nervus petrosus superficialis major to form the nervus canalis pterygoidei. The nervus canalis pterygoidei continues through the series until row 3, slide 21, is reached where the ganglion sphenopalatinum begins, the ganglion being represented by an unshaded area continuing the course of the nerve.

Bundles of sympathetic fibers and branches of the ganglion sphenopalatinum are represented by broken lines, cranial nerves and their branches by solid lines, and ganglia by unshaded areas along or within the nerves. Figures 4, 5, and 6 were drawn from sections of the same series used in making the graph, the location of these sections is indicated by the broken transverse lines. The small letters along the lines indicate bundles of nerves which are shown and similarly labeled in the figures.

Fig. 8 A graph showing the distribution of the posterior and middle palatine nerves and a part of the anterior palatine nerve to one half of the soft palate and the posterior part of the hard palate, made from the same slides which were used in making the graph shown as figure 7. The small circles indicate the approximate locations of the taste-buds and the broken line the limits of the palatal glands. The arabic numerals indicate branches of the nerves which are shown under the mucosa of the palate in figures 4 and 5. The broken transverse line marked x indicates the place of junction of the hard and the soft palate. For further explanation see the text.


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16-2
N.Max.
P.Inf.
Na.
N.Np.
Fig.6
Orb. Brs.
N.Pal. Ant.
Fig.5
G.Sp
N.Sp.
N.Pal.M.
N.Oph.
Fig.4
N.Pal. P.
G.Sem.
N.Can.Pr.
4 N.Man.
N.Abd.
G.Or.
N.P.P.
N.P.S.M.
N.Tri.
Slide 16 Row 1
Series 14
Sym.
G.Gen.
N.Pal.Ant.
N.Pal.M.
N.Pal.P.
7
8

On these nerves no encapsulated nerve endings were found. The nerves of the glands are not so well demonstrated. Bundles of fibers can be traced until they break up into smaller bundles or isolated fibers among the gland alveoli. The nerve supply of the taste-buds, with one exception, could be identified as coming from one or the other of the palatine nerves.

In the series represented in the graph the posterior palatine nerve is represented by two relatively large nerve bundles. These emerge through separate canals in the bone. One of them is directed anteriorly and supplies a small part of the lateral side of the hard palate, the other passes posteriorly to supply a limited area of the hard palate and one entire half of the soft palate. The middle palatine nerve is distributed to the lateral part of the hard palate anterior and lateral to the place of emergence from its canal. Two bundles of the anterior palatine nerve are shown, one passing medially and the other posteriorly, to supply an area of the hard palate medial to that supplied by the other nerves. Practically the entire mass of the palatal glands and all but a few of the taste-buds are located in the area supplied by the posterior palatine nerve.

In the nerves connected with the sphenopalatine ganglion there are two varieties of fibers, those of large size from the maxillary nerve, and those of small size from the sphenopalatine ganglion. In the nasopalatine nerve and in the posterior inferior nasal branch of the anterior palatine the fine fibers are grouped and leave the nerves as separate branches. These branches enter glands and are assumed to contain glandular fibers although they are not stained within the glands and could not be followed to their final termination.

In the posterior branch of the anterior palatine and in the middle and posterior palatine nerves the fine fibers are intermingled with those of larger size and terminate in company with the larger fibers. Except certain of the fine fibers which enter the walls of the blood-vessels the endings of the finer fibers could not be determined. In the area of the palate supplied by the posterior palatine nerve the fine fibers presumably supply the palatal glands, the muscle fibers in the walls of the blood-vessels,

and the taste-buds, if these structures receive their innervation from the facial nerve. From what has been said above, however, concerning the minute bundles of fine fibers from the sphenopalatine ganglion to the posterior palatine nerve, it does not seem possible that they are numerically sufficient for the supply of all of these structures. The only other possible source for their nerve supply is from the sympathetic or the trigeminal nerve.

The evidences from comparative anatomy do not materially assist in clearing up this problem. In petromyzonts, Johnston ('08) found that the maxillary nerve supplied the roof of the mouth. The palate of bony fish (Herrick, '99, '00, '01) is supplied by the ramus palatinus VII. In the amphibians Coghill ('01, '02) and Norris ('08, '13) describe anastomoses between the ramus palatinus VII and branches from the fifth nerve, the resulting nerves being distributed to the roof of the mouth, the teeth and the nasal capsule. In none of these forms is there a sphenopalatine ganglion present, although Johnston ('08) describes ganglion cells in the roof of the mouth in cyclostomes, and Norris ('08), in Amphiuma means, mentions ganglion cells at certain points on the anastomoses between the ramus palatinus VII and ramus ophthalmicus profundus V. Norris, in Siren lacertina ('13) also describes branches from the ramus platinus VII to the vessels of the roof of the mouth.

In this connection it is interesting to note that Herrick ('16, p. 243) says, "Unlike the visceral sensory system, however, its (referring to the gustatory apparatus) peripheral fibers have no connection with the sympathetic nervous system and the reactions may be vividly conscious." If this statement be literally true, then the taste-fibers of the palate must come from the trigeminal nerve. This is hardly probable, for Herrick ('01) has shown that in the siluroid fishes, where the taste-buds are very numerous, none of them are directly supplied by the trigeminal nerve. If this statement means that taste-fibers have no connection with sympathetic ganglion cells, then it is possible for the taste-buds of the palate to be supplied by fibers from the facial nerve which pass through the sphenopalatine ganglion without interruption.

In conclusion it can be said that, in the mouse, the epithelium of the palate is supplied by fibers from the maxillary nerve, and that the muscle fibers in the walls of the blood-vessels are supplied by fibers from the sphenopalatine ganglion. It does not seem possible for the taste-buds and glands of the palate to be supplied by fibers from the sphenopalatine ganglion. The evidences that they are supplied by the trigeminal nerve is equally inconclusive. In the absence of other direct anatomical observations on this problem in mammals it must, for the time being, remain an unsettled question.

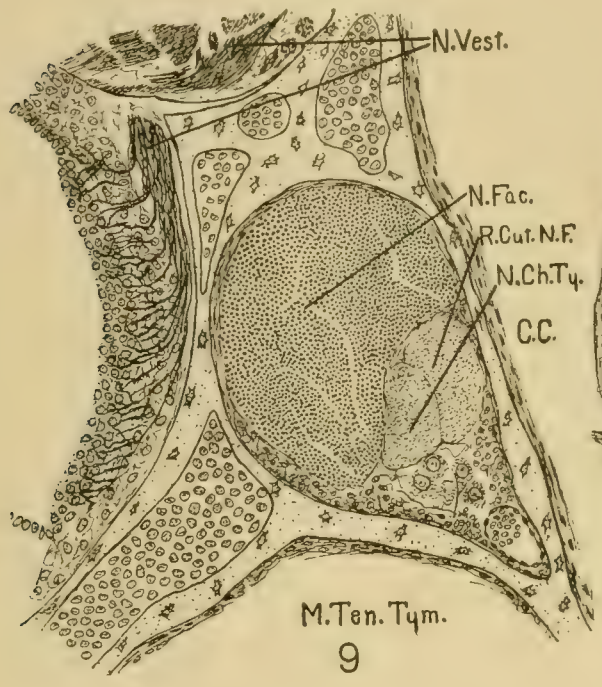
Until recently the nerve supply of the m. tensor veli palatini and the m. levator uvulae was believed to come from the facial nerve. In the mouse the branch from the mandibular nerve to the internal pterygoid muscle passes ventrally around the stapedial artery and, after supplying the internal pterygoid, sends one branch into the levator veli palatini and another which passes ventral to the otic ganglion and terminates in the tensor tympani muscle. The tensor veli palatini and the levator uvulae muscles are supplied by a branch from the accessory portion of the spinal accessory nerve which sends fibers into these muscles and terminates in the muscles of the pharnyx.

2. Nervus stapedius

The first branch of the facial nerve peripheral to the genicu ate ganglion is the nervus stapedius which arises in the dorsal wall of the tympanic cavity. In the mouse this is undoubtedly a motor nerve. The fibers composing it come from the medial side of the facial stem (figs. 10, 11, and 12, *N.Stap.*). They unite into a small bundle at the junction of the medial and dorsal borders of the facial nerve and pass into the stapedius muscle, to terminate after the manner of motor nerves elsewhere. There are no ganglion cells along its course, nor does it contain fine fibers.

Weigner ('05), in the ground-squirrel (Ziesel), describes a ganglion at the place of origin of the nervus stapedius and states that it contains many fine fibers similar to those of the nervus

intermedius. He also reports that cutting the facial nerve where it passes across the cochlea did not result in a degeneration of all of the nervus stapedius although there is a degeneration of the entire facial nerve distal to the cut. He offers no explanation for this, other than the implied one that the nervus stapedius is composed mostly of fibers arising in the microscopic ganglion located at its place of origin from the facial nerve.



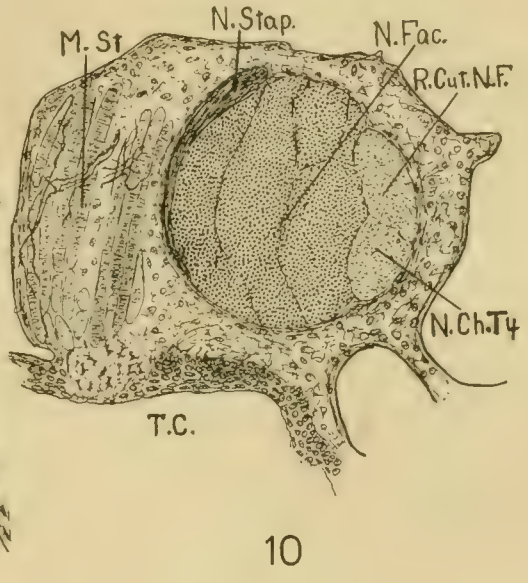


Fig. 9 Sagittal section through the nervus facialis at the lateral edge of the ganglion geniculi and medial to the genu externum showing the two bundles of fine fibers which pass from the ganglion peripherally in the nervus facialis. Same series as figures 1, 2, and 3. 225 μ lateral to figure 3. $\times$ 166.

Fig. 10 Transverse section through the nervus facialis just posterior to the genu externum and through the origin of the nervus stapedius. The two bundles of fine fibers are shown along the lateral side of the nerve. $\times$ 166.

Remembering that the mouse and the ground-squirrel both belong to the order rodentia, an order of mammals in which there is little variation in anatomical structure, I am unable to account for the differences in this nerve in the two animals.

3. Nervus chorda tympani

The chorda tympani arises from the ventral side of the facial nerve along the posterior part of the upper wall of the tympanic cavity between the origin of the nervus stapedius and the anastomosis with the ramus auricularis vagi. It is formed by the more ventral and smaller of the two bundles of fine fibers which come from the interior of the geniculate ganglion and pass peripherally as a part of the facial stem (figs. 9, 10, 11, 12, and 13, *N.Ch.Ty.*). It has been shown above that it contains fibers which are processes of cells in the geniculate ganglion and others which pass through the ganglion without interruption.

In the mouse, soon after its origin, the chorda bends medially and anteriorly and passes through a fissure-like opening into the tympanic cavity. Here it extends anteriorly in a shallow groove along the upper part of the lateral wall, and then along a groove in a spicule of bone which projects into the cavity. At the apex of this spicule the nerve crosses a small gap to reach the medial surface of the head of the malleus, across which it passes lying ventral to the attachment of the tendon of the tensor tympani muscle. In the anterior part of the cavity it passes along the medial side of the anterior process of the malleus, accompanying that process into the fissure between the tympanic and periotic bones (petrotympanic fissure) through which it extends into the infratemporal fossa. In the infratemporal fossa the chorda tympani passes medially and ventrally posterior to the emerging mandibular nerve. After a course of varying extent it joins the posterior aspect of the lingual nerve.

Just before joining the lingual there is usually an anastomosis with a bundle of fibers from another source. This is, in a majority of my series, a small branch arising from the mandibular nerve. In one series a branch from the auriculotemporal nerve joins it; in another a branch from the lingual joins it, the two then uniting with the lingual; in one series there is no anastomosis of any sort. All of the branches which anastomose with the chorda tympani are composed of fibers of larger size than those in the chorda. When followed centrally they become lost in the trunk of the mandibular nerve.

In none of the series is there a connection of any sort with the otic ganglion.

Weigner ('05) mentions an almost constant anastomosis between the lingual and mandibular nerves in man, the chorda joining the lingual just distal to it. He also describes a communication from the great superficial petrosal nerve to the fibers in the facial which form the chorda, and states that there are many scattered ganglion cells along the chorda tympani both before and after its origin from the facial. In the mouse no such communication can be identified. With the exception of one series, in which there is a small ganglion at its place of origin from the facial nerve, no ganglion cells are present in the chorda.

A. The nerve supply of the tongue and the salivary glands. In one transverse series the nerves in the tongue were carefully followed. The results of this study will be briefly presented.

The hypoglossal nerve is composed of fibers of uniform size resembling closely those in the motor portion of the facial nerve. Immediately after entering the tongue it divides into two branches which run anteriorly, one close to the septum and the other farther laterally. From these, small branches are given off in all directions, many of which were followed to their termination in motor nerve endings in the muscle fibers of the tongue.

The glossopharyngeal nerve is composed of fibers of medium and small size resembling those of the lingual nerve. It enters the tongue between the hyoid bone and the thyroid cartilage and passes anteriorly for a short distance along the side of the base of the tongue. In this part of its course there is, in all my series, a ganglion almost as large as the otic and having the histological characteristics of a sympathetic ganglion. Just anterior to this ganglion the nerve breaks up into five bundles which enter the tongue and spread out in a fan-shaped manner.

The most medial of these passes medially and posteriorly and supplies the mucous membrane of the tongue as far as the larynx. The next passes medially and anteriorly and supplies the dorsum of the tongue in the region of the single circumvallate papilla. This branch sends a number of fibers into the base of the papilla, which, together with a similar contribution from the opposite

side, supply this structure. These fibers form a plexus external to the valley and within the core of the papilla. From these plexuses fibers supply the taste-buds and the epithelium of the papilla and the surrounding mucous membrane. In two series a microscopic ganglion is present within the papilla.

The most lateral of the five branches of the glossopharyngeal nerve extends anteriorly along the lateral side of the tongue and terminates in the foliate papillae. The other two bundles are distributed to the tongue over an area extending as far forward as a line connecting the anterior limits of the foliate and circumvallate papillae.

The lingual nerve, because of the fibers in it from the facial nerve, was carefully studied. From one transverse series a graph of the course, branches, and distribution of this nerve within the tongue was made, and all the branches, as far as possible, followed to their termination.

From its union with the chorda tympani the lingual nerve passes medially, ventrally, and anteriorly to reach the tongue. It lies between the internal and external pterygoid muscles, then between the internal pterygoid and the mandible, and finally between the mylohyoid and the side of the tongue. It curves around the ducts of the submaxillary and sublingual glands, turns anteriorly lateral to the genioglossus, and inclines dorsally into the substance of the tongue.

A few branches are given off from the lingual nerve before it enters the tongue. At the ventral edge of the internal pterygoid and under the mylohyoid muscle a number of small branches are given off which pass posteriorly to the submaxillary and sublingual glands. At the ventral edge of the internal pterygoid a larger branch arises which passes medially to supply an area of the mucous membrane of the cheek opposite the folliate papillae. This branch sends fibers into the epithelium, into a mass of glands, and into a number of taste-buds (eight in the series studied). Along the side of the tongue, in relation to the gland ducts, one relatively large and a number of smaller branches are given off which pass forward in company with the ducts and supply the mucous membrane of the floor of the mouth and the gum behind the lower incisor teeth.

In the tongue the lingual nerve passes forward midway between its lateral edge and the median septum. One branch is given off immediately after the nerve enters the tongue. This passes dorsally and posteriorly to supply the area in front of that supplied by the glossopharyngeal nerve. The remaining branches arise irregularly and supply all of the tongue in front of this area. Some of the branches extend laterally, some to the ventral surface, but the larger and most numerous branches pass to the dorsal surface. In the ventral part of the tongue a few small branches join those of the hypoglossal nerve, and a few others end in an undetermined manner among the muscle fibers. The majority of the branches, however, could be followed to the mucous membrane.

In the series studied the taste-buds are located in both walls of the valley around the circumvallate papilla, in the foliate papillae, in a small area of the cheek opposite the foliate papillae, and irregularly scattered over the dorsal surface of the tongue in the area supplied by the lingual nerve. There are no scattered taste-buds in the area supplied by the glossopharyngeal nerve nor along the sides or the ventral surface of the tongue. In the area supplied by the lingual nerve there are thirty-six taste-buds in one-half of the tongue. These are widely separated posteriorly and become more numerous as the tip of the tongue is approached. Each of them is placed near the surface of the epithelium on the top of a flat tunica propria papilla.

One or two bundles of nerve fibers from the lingual nerve were followed into the bases of the papillae on which the taste-buds are located. In the papillae these fibers break up into a number of branches and form an intricate complex of very fine fibers. From these plexuses some fibers pass into the taste-buds. Other fibers pass into the surrounding mucous membrane, so that the taste-bud and a considerable area of the mucous membrane is supplied from the plexus in each papilla.

The most striking feature of the nerves within the tongue is the large number of ganglia and ganglion cells along them. The majority of these cells are in clumps or microscopic ganglia along the nerve bundles. Along the glossopharyngeal nerve there are

six of these ganglia, along the lingual nerve they are far more numerous, being forty-one in number. Along the hypoglossal nerve there are scattered smaller ganglia and isolated cells. Fibers from the lingual, the glossopharyngeal and even the hypoglossal nerve can be followed into these ganglia to end in an undetermined manner among the ganglion cells.

A count of the cells in these ganglia along the lingual and hypoglossal nerves was made. Only those in the ganglia in which there is a definite nucleolus were counted, so that the figures obtained are less rather than more than the actual number present. In this one series, in one-half of the tongue, there were 1079 cells, 957 along the lingual and 122 along the hypoglossal nerve.

While the presence of ganglion cells in the tongue has long been known, I have failed to find a reference as to their number or their significance. They are usually dismissed with the statement that they are sympathetic cells. I am of the opinion that much will be added to the knowledge of the nerve supply of the tongue when the central connections, the endings of the fibers, and the functions of these ganglion cells have been worked out.

In this work nothing has been found other than in support of the generally accepted view of the nerve supply of the tongue. That the taste-buds are supplied by the chorda tympani has long been known. The course of the taste-fibers into the brain was, however, for a time, an unsettled question. From clinical cases evidences have been deduced in support of every possible pathway for these fibers into the brain stem. Many of these are included in the review of the literature in the articles by Cushing ('03), Weigner ('05), and Sheldon ('09).

The carefully conducted experiments of Cushing ('03) have done much to prove that the taste-fibers enter the brain over the nervus intermedius. In the mouse the anatomical evidence supports this view. No connection, such as was found by Weigner ('05) between the chorda tympani and the great superficial petrosal nerve is present, and the entire absence in all my series of a communication from the facial nerve to the tympanic plexus excludes even the possibility that the taste-fibers reach the brain except through the nervus intermedius.

Cushing ('04) has presented very convincing clinical evidence that the chorda tympani supplies the tongue with certain forms of common sensation. After trigeminal neurectomy he found that sensations of pain and temperature and tactile sensations are absent in the area supplied by the lingual nerve. There remained, however, the ability of the patient to appreciate the presence, the general location, and the movement of a piece of cloth or a cotton swab across this area.

The nerve supply of the submaxillary and sublingual glands in the mouse corresponds closely to that given by Langley ('90) and Huber ('01) for the dog and cat. In the mouse the sublingual or the retrolingual gland is located lateral and ventral to the submaxillary and is pure mucous in type while the submaxillary is pure serous.

The nerves to these glands leave the lingual as a number of branches (five to eight) arising deep to the mylohyoid muscle and rather widely separated. These nerves come into relation with the ducts and pass with them into the glands. Along these nerves there are several small ganglia. In the series most carefully studied there were five of these, two sending their fibers along the submaxillary duct, one along the sublingual duct, while the other two send fibers along the ducts into both glands. Within the submaxillary gland there are two large ganglia and several smaller ones. The ganglion cells in these ganglia are so numerous that if each is supplied with a basket-work from the chorda tympani as described by Huber, each chorda fiber must divide and terminate in relation to several cells. A fiber dividing and ending in relation with two cells was seen by Huber.

In the mouse the fibers from the sympathetic to these glands are much more numerous than those from the chorda tympani. An estimate, based on the cross-section area of the nerves from the two sources, shows the fibers from the sympathetic to be at least ten times as numerous as those from the chorda.

Most of the sympathetic fibers enter the glands along the arteries. One relatively large and constant bundle, however, enters the dorsal border of the submaxillary gland anterior to the hilus and joins one of the main subdivisions of the duct. There

are no ganglia on the sympathetic nerves into which fibers from the chorda could not be traced.

4. Ramus cutaneus facialis and the anastomosis with the ramus auricularis vagi

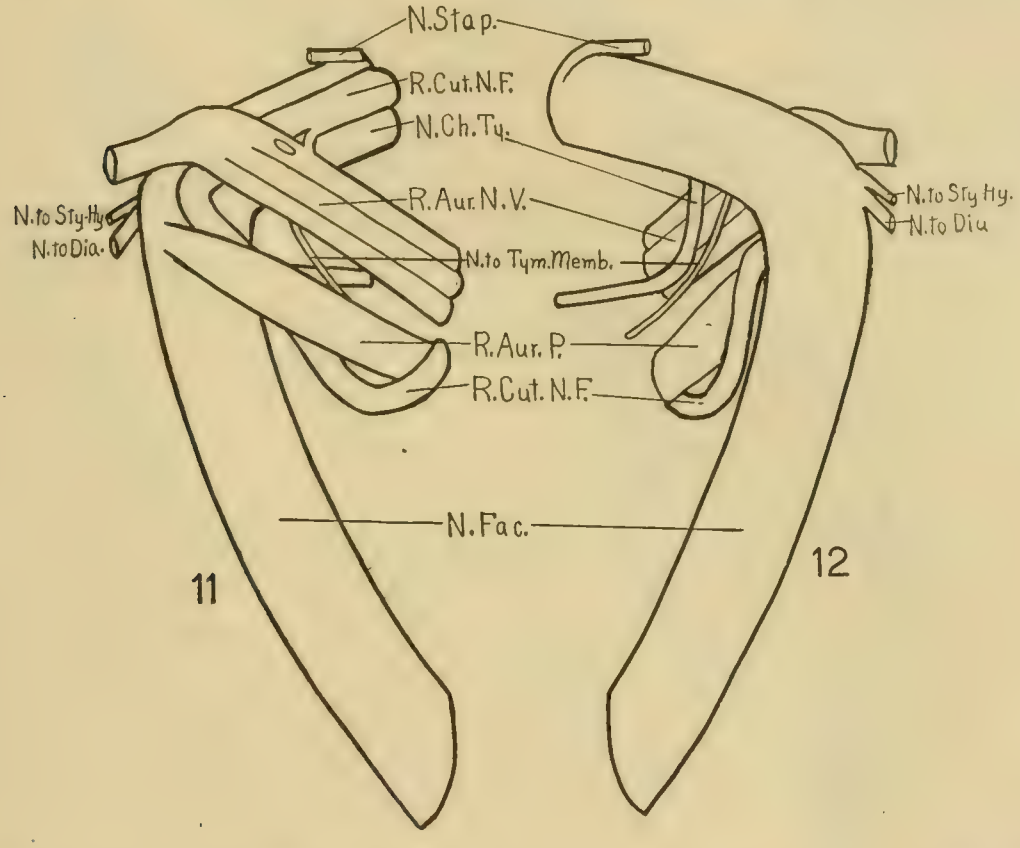
A short distance distal to the origin of the chorda tympani, at the most posterior part of the bend of the facial nerve around the tympanic cavity, the ramus auricularis vagi anastomoses with the facial nerve and the remainder of the branches of the facial arise. These branches are the nervus auricularis posterior, the nerves to the posterior belly of the diagastric and stylohyoid muscles, and a branch which had a cutaneous distribution to the skin of the auricle. This nerve I have called the ramus cutaneus facialis. Because of the intimate relationship between the cutaneous branch of the facial and the auricular branch of the vagus, these nerves will be described together.

The fibers which form the cutaneous branch of the facial nerve are those of small size from the geniculate ganglion which pass peripherally in the trunk of the facial nerve forming the more dorsal and larger of the two bundles of fine fibers described above (figs. 9 and 10, *R.Cut.N.F.*). The auricular branch of the vagus arises from the cells of the ganglion jugulare and passes anteriorly and laterally posterior to the tympanic cavity to reach the lateral side of the facial nerve. No communication to it from the glossopharyngeal nerve was seen.

While the relations between the fibers from different sources are rather complicated at the anastomosis, it is possible, because of the difference in the size of the fibers, to trace them from section to section. The motor fibers of the facial nerve are the largest, those from the geniculate ganglion the smallest, and those from the vagus intermediate in size. From one transverse series a model of this region was made. Outline drawings of this model are shown as figures 11 and 12.

The auricular branch of the vagus crosses the lateral side of the facial nerve and breaks up into four small bundles (*R.Aur.N.V.*). The cutaneous fibers of the facial pass under the fibers from the

vagus to leave the facial stem as a separate branch just distal to the anastomosis (fig. 11). Fibers from the bundle in the facial stem pass into the auricular branch of the vagus proximal to the anastomosis. At the anastomosis there is an interchange of fibers between the auricular branch of the vagus and the cutaneous fibers in the facial stem. This interchange is shown in figure 13.



Figs. 11 and 12 Outline drawings of a blotting-paper model of the nervus facialis showing its relation to the ramus auricularis vagi and the branches arising above and behind the tympanic cavity. Figure 11 is a lateral and slightly posterior view of the model; figure 12 a medial and posterior view of it. × 33.

After the cutaneous branch of the facial and the auricular branch of the vagus have separated from the facial stem, each nerve contains fibers from the other. Because the greater proportion of the fibers of one nerve come from the facial, it is considered as a branch of that nerve, the other for the same reason being considered as a branch of the vagus.

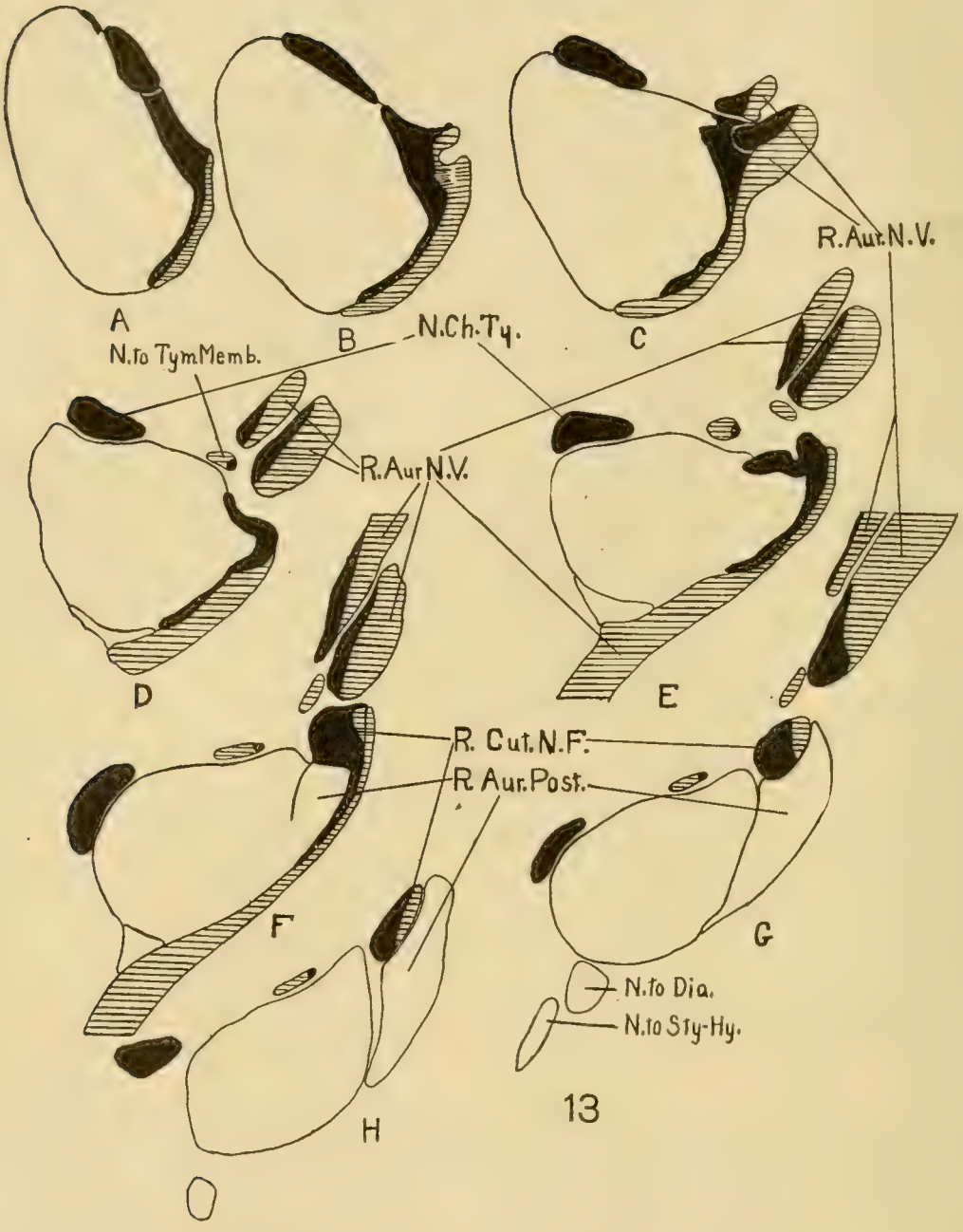


Fig. 13 Outline drawings of every other section through the nervus facialis in a horizontal series, beginning above and passing ventrally through the anastomosis with the ramus auricularis vagi, to show the mixing of the cutaneous facial fibers with those from the vagus. The motor facial fibers are unshaded, the fibers from the ganglion geniculi are black, and the vagus fiber cross-hatched. × 100.

In different series there are some differences in the arrangement of these nerves. In one series the ramus auricularis vagi is composed of a single large bundle, in another the ramus cutaneus facialis is composed, after its origin, of two bundles, and there are apparently some differences in the number of fibers which interchange. However, the essential arrangement is constant and as described above.

Immediately after its origin the ramus cutaneus facialis passes from the medial to the lateral side of the posterior auricular nerve. From here it extends anteriorly and laterally above the external auditory meatus until the point of the attachment of the cartilage of the auricle to the side of the cranium is reached. At this point it enters the auricle, extending upward and anteriorly along the medial side of that part of the auricle which bounds the pouch-like concha laterally (figs. 14A and 14B). It gives off branches in this region which supply the skin and hair follicles. Other branches pass dorsally beyond the concha and supply the skin of the posterior third of the lateral surface of the auricle.

The ramus auricularis vagi sends a small branch to supply a part of the external auditory meatus and the tympanic membrane, this branch containing a few fibers from the facial (fig. 13D). Otherwise the nerve supply of the tympanic membrane is the same as that given by Wilson ('07, '10–'11). After the origin of the branch to the tympanic membrane the auricular branch of the vagus passes along the cranial side of the concha. It supplies this area of skin and its terminal branches pass dorsally beyond the concha to supply the anterior two-thirds of the lateral surface of the auricle (fig. 14).

These two nerves have been followed with great care from their origin to their place of ending. Each, in that part of its course where it is related to the auricular cartilage, passes between the cartilage and the skin, a location where there are no muscle fibers. Branches of each have been followed to their final ending and have been found to end in a plexiform manner immediately under the epithelium or around the hair follicles. It is safe to conclude that they are both common sensory in function for the supply of the skin and hair of the auricle.

The anatomical and clinical evidence for the presence of general cutaneous fibers in the nervus facialis is meager in amount and inconclusive. In petromyzonts, Johnston ('08) describes general cutaneous fibers arising from the geniculate ganglion and passing to the ventrolateral surface of the head below and behind

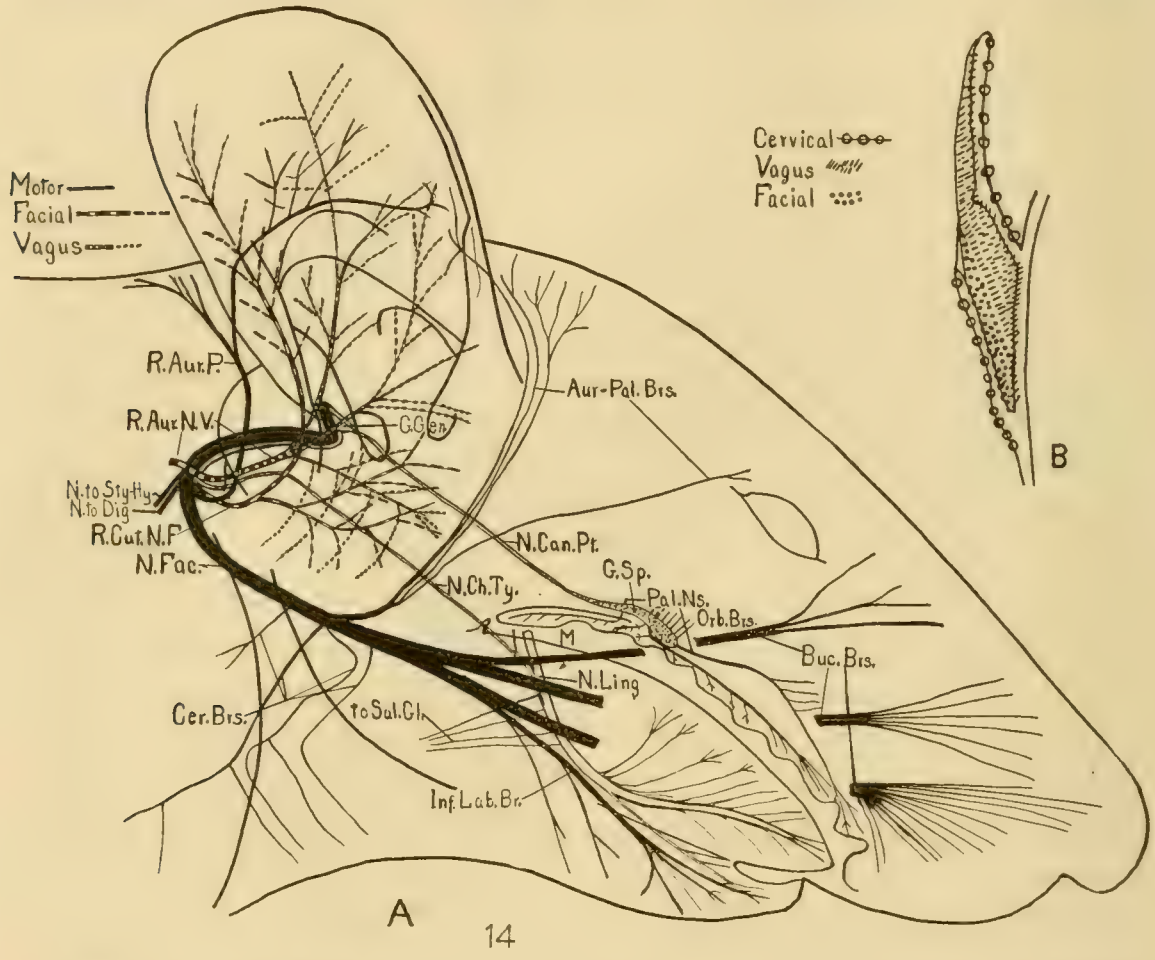


Fig. 14 A A diagram of the connections and distribution of the nervus facialis of the albino mouse, based largely on a flat reconstruction made by projection of sagittal sections on to a median plane.

14 B A diagram of a coronal section through the auricle to show the areas of skin supplied by the vagus, facial and cervical nerves.

the orbit. In bony fish, Herrick ('99, '00, '01) found a general cutaneous component in the facial nerve for the region of the operculum, the fibers, however, being derived from the Gasserian ganglion. In the amphibia, Norris ('13) found a general cutaneous component in Siren, and Herrick ('14) described fibers from the geniculate ganglion in Amblystoma larva which enter the

spinal V tract, this tract being considered as a tract whose nucleus is generally cutaneous in function.

Van Gehuchten ('98) found that a few cells of the geniculate ganglion degenerate after cutting the facial nerve at its exit from the facial canal. In an abstract of an article by DeGaetani ('06) sensory fibers in the ramus temporofacialis are mentioned. Weigner ('05) describes fibers of small caliber, presumably intermedius fibers, in the facial nerve of man distal to the origin of the chorda tympani.

The chief clinical evidence for the presence of such fibers in the facial nerve has been presented by Hunt ('15). By a method which he calls the herpetic method and which is based on the fact that herpetic vesicles on the skin are caused by pathological processes involving the ganglion from which the cutaneous fibers arise, he attributes the supply of certain parts of the auricle, including the concha, to the facial nerve. One of the probable pathways to the skin for these fibers which he mentions is through the auricular branch of the vagus. In the different cases reported the areas showing the herpetic eruption vary to some extent. If homologous conditions are found in man to those here reported in the mouse this variation can probably be accounted for by the mixing of the vagus and facial fibers at the point of the anastomosis.

In the mouse there is no evidence that either the inner or middle ear receive fibers from the facial nerve. A communicating branch from the facial nerve to the tympanic plexus is not present in any of my series.

5. Nervus auricularis posterior and the nerves to the stylohyoid and digastric muscles

The posterior auricular nerve arises from the lateral side of the facial nerve just distal to the origin of the ramus cutaneus (figs. 11 and 12, *R.Aur.P.*). It passes dorsally behind the auricle and is distributed to the muscles posterior to and along the cranial side of the auricle. It is composed of fibers from the motor part of the facial and is a purely motor nerve. A similar area of skin of the auricle is supplied by sensory fibers from the second and third cervical nerves.

From the most posterior part of the convexity of the facial nerve, and just distal to the anastomosis with the ramus auricularis vagi there arise from the motor part of the facial nerve two small motor nerves which are distributed to the posterior belly of the digastric and the stylohyoid muscles (figs. 11, 12, and 13G, *N. to Dia.*, and *N. to Sty-hy.*).

SUMMARY AND CONCLUSIONS

The facial nerve of the mouse corresponds very closely to that of other mammals and man, and more closely resembles the glossopharyngeal than any of the other cranial nerves. It consists of two parts: one part made up of those fibers which arise in the motor nucleus and form the motor part of the nerve, the other part being formed by the nervus intermedius and its peripheral continuation.

The motor part of the facial nerve supplies the stapedius, the stylohyoid, the posterior belly of the digastric, the auricular muscles, and the superficial muscles of the face including those of the vibrissae (special visceral efferent component; Herrick, '16, p. 146).

The nervus intermedius is composed of afferent fibers having their cell bodies in the ganglion geniculi, and efferent fibers which have no connection with the cells of the ganglion. The ganglion itself is of the cerebrospinal type of ganglia and is composed of unipolar ganglion cells.

The nervus intermedius has three branches, the great superficial petrosal nerve, the chorda tympani, and the ramus cutaneus facialis. The first two contain both afferent and efferent fibers the third is composed entirely of afferent fibers. The efferent fibers which enter the great superficial petrosal nerve form a separate efferent root of the nervus intermedius.

The great superficial petrosal nerve enters the sphenopalatine ganglion. Sympathetic fibers from the superior cervical sympathetic ganglion reach the sphenopalatine ganglion by way of the deep petrosal nerve and the nerve of the pterygoid canal, and through the nervus abducens. Because the termination of these fibers in the sphenopalatine ganglion has not been determined, the function of each set of fibers is uncertain.

Fibers from the sphenopalatine ganglion terminate in the gland of Stenson, in the medial part, at least, of the lacrimal gland, in the glands of the nasal septum, and along the blood-vessels of the nose and in the palate (general visceral efferent component). If fibers of the great superficial petrosal nerve supply the taste-buds of the palate, which, in the mouse is by no means certain, they probably pass through the sphenopalatine ganglion without connection with the ganglion cells. The nerve supply of the glands of the palate could not be determined.

The afferent fibers in the chorda tympani carry taste impulses from the anterior part of the tongue (special visceral afferent component). The efferent fibers of the chorda tympani terminate in the ganglia in connection with the submaxillary and sublingual salivary glands, each fiber ending in relation with more than one ganglion cell. There is also some clinical evidence that the chorda tympani contains afferent fibers which carry impulses of certain kinds of common sensation from the tongue.

The afferent fibers of the ramus cutaneus facialis terminate in the skin of the external auditory meatus, in the skin of a part of the auricule and possible a part of the tympanic membrane (general somatic afferent component). Fibers from the ramus auricularis vagi are distributed as a part of this nerve, and cutaneous fibers from the facial nerve are distributed through the ramus auricularis vagi.

There are several unsolved problems in connection with the anatomy and function of the mammalian facial nerve which have been suggested by this work. Among these may be mentioned: 1) The presence and the termination of cutaneous fibers in the facial nerve of larger mammals and man. 2) If such be found present, the central connections of the cutaneous fibers. 3) The termination of the afferent and efferent facial fibers and the sympathetic fibers of the nervus canalis pterygoidei in the sphenopalatine ganglion and the function of each set of fibers 4) The nerves within the tongue, especially the termination of the fibers and the significance of the large numbers of ganglia and ganglion cells along the nerves.

It is hoped that in the near future the time and the material will be available for the working out of some of these problems.

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Resumido por el autor, Kiyoyasu Marui.

Sobre la fina estructura de la sinapsis de la célula de Mauthner, con especial mención de la "red de Golgi" de Bethe, los "piés nerviosos terminales" y la "red terminal nerviosa pericelular" de Held.

A pesar de numerosas investigaciones la estructura de la sinapsis de una célula nerviosa no está completamente aclarada y todavia se necesita una investigación cuidadosa y exacta, basada en material adecuado. El autor ha estudiado la fina estructura de las sinapsis de la célula de Mauthner de los peces óseos, empleando diferentes métodos, e intenta explicar la naturaleza de la " red de Golgi" de Bethe, su relación con las estructuras nerviosas pericelulares, y la estructura de los "pies nerviosos terminales." También intenta dar una explicación acerca de la existencia de la "red nerviosa pericelular" de Held y la teoria de los contactos o continuidad de las neurofibrillas. Los resultados de estas investigaciones pueden resumirse del siguiente modo: 1) La red de Golgi es de naturaleza gliar. 2) La parte no medulada de las fibras nerviosas está envuelta por una vaina de tejido gliar cuya fina estructura se desconoce. 3) La red nerviosa pericelular terminal no es realmente una red sino una imágen producida por el teñido simultáneo de la red de Golgi. 4) La teoria de los contactos es una imposibilidad histológica; la continuidad de las fibrillas intra y extracelulares pudo comprobarse claramente. 5). Los piés terminales no son órganos específicos de contacto, sino puntos del trayecto de las fibras axónicas en los cuales tiene lugar una disolución de dichas fibras. 6). No ha podido observar el autor estructura reticular en los piés terminales ni en la célula de Mauthner.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 9

ON THE FINER STRUCTURE OF THE SYNAPSE OF THE MAUTHNER CELL WITH ESPECIAL CONSIDERATION OF THE 'GOLGI-NET' OF BETHE, NERVOUS TERMINAL FEET AND THE 'NERVOUS PERICELLULAR TERMINAL NET' OF HELD

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FIFTEEN FIGURES

INTRODUCTORY NOTE

The problem of the synapse and the transmission of stimulus from one nerve cell to another has been a topic of study for more than a century; we can, however, say in the retrospect that it was really impossible to come to any safe result on this subject before the discovery of Golgi's method. Although recent studies by many investigators by means of different methods (Apathy, Bethe, Held, Cajal, Bielschowsky) have brought forth many a valuable contribution, we are not yet fully enlightened in many respects. On the basis of their exploration by means of the Golgi method, Golgi and his pupils came to the hypothesis that the gray matter of the central nervous system contains a continuous mesh-work, which consists of the telodendria of the axone and of the initial collaterals of the cells of the first type and of the collaterals from tracts. Forel (9), Cajal (12, 13), Kölliker (9), Retzius (26), and others denied the existence of this net-work on the strength of their observations. They declared that the Golgi net is a false net-work, which in fact is a kind of felt-work made of the dense processes of neighboring nerve cells.

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The embryological study of the nervous system supported this idea, inasmuch as it was believed that the growth of the axis-cylinder from the cell-body of an embryonic ganglion cell could be pursued for some distance. Waldeyer (9) summed up all the facts obtained by means of the Golgi technic in 1891 and formulated the 'neurone theory,' in which he took for granted that the nervous system is composed of numerous nerve elements, which are independent anatomically as well as genetically. This theory implied the so-called 'contact theory;' according to the latter, the telodendrion of the neurone terminates with free endings and the conduction of a stimulus from one neurone to another takes place by means of contact between the nerve endings and the nerve cells.

Apathy (9), who demonstrated by his own method the neurofibrils in clear and sharp pictures, assumed that the neurofibrils at certain points of the central nervous system cross the border of the nerve cell and through abundant splitting and anastomoses with the neurofibrils from the adjacent nerve cells form a real net-work ('neuropil'). Bethe (7), who by means of his molybden method stained the neurofibrils in the nervous system of the vertebrate, found on the surface of the ganglion cell as well as its dendrites a fine net-work of irregular configuration, which he called 'Golgi's net' in honor of the discoverer. He claimed an analogy of this net structure with the 'neuropil' of Apathy in the invertebrate, and interpreted it as the connecting link between the ganglion cells and the nerve fibers, which come afar from other cells. He claimed to have found that on the one hand the nerve fibers go over to this pericellular net-work with their telodendria and on the other hand the intracellular neurofibrils enter this same net structure. These theories of Apathy and Bethe cannot therefore be harmonized with the 'neurone doctrine' in which the nerve fibers are considered as non-anastomosing. It is to be added here that a pericellular net-work continuous with the nerve fibers was described also by Auerbach (2, 3), Semi Meyer (23) (24), and Held (17, 18). But the first two stood on the standpoint of neurone and contact theory, while

the last on the basis of his investigations accepted his doctrine of double interneuronal continuity. According to Held, the pericellular ends of axis-cylinders are characterized by the loosening of their axospongium and the densely embedded 'neurosomes' and the so constituted axis-cylinder-ends ('Achsencylinderendfläche') are connected with the protoplasm of the nerve cell, which is covered by them, so closely, that there exists no contact but a continuity between the two. Moreover, Held assumed that the different axis-cylinders, which enter together in the nervous cover of a ganglion cell, do not lie isolated side by side, but are combined into a continuous net-work, which he called the pericellular nervous terminal net (perizelluläre nervöse Terminalnetze'). He believed that also by means of Golgi's method he could obtain this 'pericellular terminal net,' which covers widely the cell-body as well as its processes and seemed to receive numerous axis-cylinders to its beams. When Held ('97; 17) demonstrated for the first time this silver-impregnated mesh, he identified it at first with the Golgi net, which Golgi produced with the same technic and interpreted as a neurokeratin cover of the nerve cell. Later on (17) he changed his opinion and then considered the Golgi net as not identical with his nervous net. He asserted that the 'Golgi net,' which was demonstrated by Golgi, Semi Meyer, and then by Bethe, was probably not of a nervous nature and denied the direct connection of this net-work with the nervous elements. On the contrary, he was of the opinion that two kinds of net-work—the Golgi net and the pericellular nervous terminal net—occupy the surface of the nerve cell, and that alternately. He transferred his 'neurosome-conglomerations' into the mesh of the Golgi's net and claimed to have found that these conglomerations are connected into a net-work by minute anastomosing bridges, which extend between them, over or beneath the beams of the Golgi net. He also figured the direct connection of the axis-cylinders with these neurosome-conglomerations. As regards the formation described by Auerbach, Held assumed that he observed the same structure.

Ramón y Cajal ('03, 12) summed up the results of his inves-

tigations by means of his own technic and still adhered to the contact theory. According to the Spanish author, Apathy's elementary grating ('Elementargitter') does not exist; the 'neuropil' of the latter is not a three dimensional net-work, but is to be regarded as a plexus composed of delicate processes of ganglion cells. We should emphasize here that Retzius (26) could not confirm the 'Elementargitter' even in Apathy's own preparations. In opposition to Bethe's hypothesis of the independence and the individuality of the neurofibrils, Cajal claimed to have observed a neurofibril reticulum in the nerve cells, which is said to be especially clear at the surface of the cell and around the nucleus. The Golgi net did not appear in his new preparations; moreover, his study by the Bethe's technic showed him a direct conjunction of the Golgi net with the 'Füllnetz' of Bethe, which the latter interpreted as a coagulation product. On the strength of these facts Cajal came to the conclusion that the Golgi net is nothing but an artificial product formed by the precipitate from the lymph in Obersteiner's pericellular space. He denied also the transition of the axone-fibrils into the intracellular fibrils; according to him, the axones end on the surface of nerve cell in the form of knobs, and there exists always a thin layer of protoplasm, which is free from neurofibrils at the edge of the cell between the knobs and the intracellular neurofibrils. He considered this fact as a new argument of the contact theory. Held's (19, 20) observations by Cajal's method, however, brought to light two different things, namely, the reticular fibrillous structure of the knobs themselves and the continuity between the axone-fibrils and the fibril net-work of the ganglion cell. Holmgren (22) confirmed these findings of Held. Cajal (13) also figured later the reticular structure of the knobs.

By means of his method, Bielschowsky (8, 9, 10) reached a conclusion similar to that of Bethe; in the greater majority of cell types he could confirm Bethe's description of the isolated course of neurofibrils. In the other types, however, he demonstrated clearly a net-structure of the fibrils, which Bethe also admitted in some types of the nerve cells. Bielschowsky could not

acknowledge Cajal's supposition of a constant existence of a net-structure, and he attributed this to a fault of Cajal's method. With regard to the central endings of axones he formed an idea that the contact concept must come from the imperfect staining of the structure referred to. Like Held, he recognized the recticular structure of the knobs and also believed he could demonstrate neurofibrils entering the cell so as to constitute a continuity of the neurones. In addition to this fibril continuity Wolff emphasized recently the existence of the plasmatic continuity between nerve fibers and nerve cells. Bielschowsky and Wolff (11) observed also a pericellular net-work, which resembles the 'Golgi net.' They considered that it is formed by the mutual communication of the fibrillous and plasmatic substances of the nerve fibers and stated their opinion that it is probably a structure identical with the Golgi net.

According to the accurate investigation by Held (18, 19), the Golgi net is directly continuous with the 'Füllnetz' and they are both of glious nature. Economo (15), Paladino (25), and others confirmed this. As I mentioned above, Held (18) supposed the existence of two kinds of pericellular net-work. Economo (16) agreed with him. But this problem is not at all altered because Heidenhain (16) described recently that he would consider the Golgi net an artefact rather than a glious element. With regard to the pericellular terminal net, he admitted so far only that occasionally there exist sling-like loops within the arborization of one and the same branch of nerve fiber.

Despite many investigations, the theory of contact still opposes the doctrine of the neurofibril continuity. Hedenhain (16) said that one can undoubtedly deny the latter, declaring that the figures by Held (17, 19, 20), Holmgren (22), Bielschowsky (9), and Wolff (27) prove nothing at all and that with others (Cajal (12, 13), Dogiel, (14), Retzius (27), Lenhossek (cited in 16)) he sought in vain the continuity of nerve fibers and the cells in question. In 1915 Bartelmez (4) studied the Mauthner cell of teleosts, which had been investigated by Mayser[1] and Becarri (5), and

[1] Zeitschrift f. wissenschaft. Zoologie, Bd. 36, 1882.

he further analyzed the elements of the peculiar synapse of this giant cell. Lately I had the opportunity to do some experimental pathological study on this wonderful cell and its synapse, the results of which will appear soon. Careful and thorough investigations led me, however, to many interesting conclusions in regard to the structure of the synapse and the mode of conjunction of nerve cells; I will therefore describe these results in the present paper with special considerations of the condition in higher vertebrates. I beg to express here my gratitude to Dr. Adolf Meyer for his constant help and frequent advice in this work.

MATERIAL AND METHODS OF STUDY

The present work is based upon the investigation of serial sections of brains of adult Ameiurus nebulosus and Carassius auratus. The fish were decapitated, bled, and the brains were dissected out carefully but quickly, and placed promptly in different fixatives; the methods of preparation used are as follows:

At first I mention the toluidin-blue preparation, in which brains were fixed in 95 per cent alcohol for twelve to twenty-four hours. Paraffin sections 8 to 10 μ thick were stained in 1 per cent warm aqueous solution of toluidin blue, differentiated in alcohol and sometimes counterstained with eosin.

Other brains were fixed in formol-Zenker fluid twelve to twenty-four hours, cut at 8 μ in paraffin, stained in a saturated solution of thionin in a 1 per cent aqueous solution of carbolic acid and counterstained with eosin after differentiation in alcohol.

For Cajal preparations brains were placed twelve to twenty-four hours in alkaline alcohol (96 per cent alcohol with 1 per cent ammonium hydroxid). The fixed brains were then rinsed with 95 per cent alcohol, placed in distilled water till they sank to the bottom of the container and then they were placed in the silver-bath of 37° to 40°C. for ten to fourteen days, according to the size of the pieces. As silver-bath I used a 1 per cent silver-nitrate solution for the first seven to ten days, with one change, and then a 2 per cent solution with one change, in which the pieces were kept three to four days. They were then rinsed with distilled water and developed twelve to twenty-four hours in 1 per cent pyrogallic acid solution in 5 per cent formalin, again rinsed with water, dehydrated and cut in paraffin 5 to 10 μ thick. This always gave good results; in my experience the Mauthner cells are very apt to show shrinkage space after fixing in acetic alcohol for a short time (Bartelmez) (4), which is unfavorable for the study of the synapse.

I next applied the Levaditi method for spirochaeta pallida to the fish brain. The brains were fixed in 10 per cent formol twelve to

twenty-four hours, rinsed with water, placed in 95 per cent alcohol for twenty-four hours, and then placed in distilled water, till they sank to the bottom and they were then put into the silver-bath for five to seven days (in a 1 per cent silver solution for three to four days and in a 2 per cent solution for two to three days). For the reduction of the impregnated silver a 2 per cent pyrogallic acid solution in 5 per cent formol was used. The sections were cut at 5 to 10 μ. This preparation offered excellent pictures of the synapses and led to many interesting findings, which will be described later.

The Bielschowsky method of silver reduction also yielded nice pictures of the intracellular neurofibrils and the synapse. The brains were fixed in 10 per cent formol for twenty-four hours, rinsed with water, dehydrated and cut in paraffin at 5 to 10 μ. The paraffin was removed with xylol and alcohol and the sections were thoroughly washed with distilled water and placed in a 2 per cent silver solution for two to three days. The sections were treated on the slide, following exactly the directions of Bielschowsky. Some of the series were counterstained with eosin.

For Heidenhain preparations, brains were fixed in Zenker's fluid or in formol-Zenkr fluid. Some brains were put into the latter, after being fixed first in 10 per cent formol for twelve to twenty-four hours. The sections of 5 to 8 μ were stained in the iron-hematoxylin of Heidenhain. This method gave not only a clear picture of the cell-body and the synapse, but also a blue stain of the myelin sheaths that was sometimes very advantageous.

In addition to these preparations I prepared also a few series with Held's neuroglia stain, Weigert's stain, and Mallory's stain. In all I studied ninety-seven series of normal fish brains in the different methods, classified as follows:

(1)	5 series of Ameiurus nebulosus 5 series of Carassius auratus	Fixed in 95 per cent alcohol, and stained with toluidin-blue and sometimes eosin.
(2)	5 series of Ameiurus nebulosus 5 series of Carassius auratus	Fixed in formol-Zenker fluid, stained with thionin-eosin.
(3)	5 series of Ameiurus nebulosus 11 series of Carassius auratus	Cajal preparation.
(4)	14 series of Ameiurus nebulosus 15 series of Carassius auratus	Levaditi's method.
(5)	10 series of Ameiurus nebulosus 11 series of Carassius auratus	Bielschowsky's stain.
(6)	5 series of Ameiurus nebulosus 4 series of Carassius auratus	Heidenhain preparation.
(7)	2 series of Carassius auratus	Held's neuroglia stain.
(8)	2 series of Ameiurus nebulosus	Mallory's stain.
(9)	2 series of Ameiurus nebulosus	Weigert's stain of myelin sheath.

NEUROFIBRIL STRUCTURE OF THE MAUTHNER CELL

It is not the purpose of the present work to study the position, general relations, and size of this wonderful cell. My main purpose lies, on the contrary, in the further investigation of the finest structure of this giant cell. I merely refer here to the works of Mayser, Beccari (5), Herrick,[2] Bartelmez (4), and others in regard to those points. As far as the internal morphology of the Mauthner cell is concerned, it would not do to ignore the condition of the intracellular neurofibrils in the study of synapse, since, as I mentioned above, the relation between extra- and intracellular neurofibrils is not clearly decided yet. Though Bartelmez and others described the neurofibril structure of this cell, I will add the results of my study here, as there are many points not sufficiently clear.

The Cajal, Levaditi, and Bielschowsky preparations were chiefly used in my study of the neurofibril structure of the Mauthner cell. By means of the first two methods the neurofibrils were exceedingly clear in many cases, but in other cases they proved very uncertain in their results. The cell was now and then stained merely a diffuse yellow or brown, without any neurofibrils being differentiated in it, and in other cases the ground substance of the cell was also tinged more or less intensive yellow or brown, so that the neurofibril structure did not present itself clearly enough. In comparison with them, the Bielschowsky technic offered always excellent results; I shall therefore speak mainly of the Bielschowsky preparations, besides giving consideration to the best preparations of Cajal and Levaditi. Owing to the wealth of neurofibrils in the Mauthner cell, the sections must be thin in order that the course of the individual neurofibril could be followed distinctly.

The results of my investigation on the neurofibril structure in Mauthner's cell harmonize in main features with those of Bethe (6, 7), Bielschowsky (8, 9, 10), and Economo (15), in so far as the neurofibrils do not form a real net-work in the cell. On the

[2] Journal of Comparative Neurology, vol. 24, 1914, p. 343.

strength of his study by means of his method, Bethe came to the conclusion that the neurofibrils pass generally through the cell-body without any net-formation. With regard to this, however, the cells of the Ammon's horn and the Purkinje's cell remained uncertain to him. As far as the cells of the spinal ganglia and of the lobus electricus of Torpedo are concerned, he was quite sure that there exists a net-work in them. According to Bielschowsky, who worked with his method, the neurofibril structure corresponds in essential features to that of Bethe. Economo also demonstrated neurofibrils which do not anastomose with the other fibrils. Like Bethe, he found also fibrils which run isolated from one branch of one dendrite to another and are not continuous with those in the cell-body.

As figure 1 shows, the neurofibrils run straight or winding, but smoothly through the cell-body and, generally speaking, parallel to the axis of Mauthner's cell, without any anastomoses for a long distance. Some of the neurobrils can even be followed through the whole length of Mauthner's cell, without any bifurcation of neurofibrils. The latter occurs, after all, only now and then, and this strongly suggests that the neurofibrils do not form a real mesh-work in the cell. On this point I certainly agree with Bethe (6), who argued that bifurcation would appear oftener, if there were really a net-work in the cells. Economo (15) suggested that there probably occurred merely a sticking together of neurofibrils, which lie side by side in the cells. In the neighborhood of the nucleus the course of the fibrils is slightly more irregular, but I could not observe any anastomosis between the neurofibrils. At the starting point of the cell processes the fibrils radiate forth into the cell-body and at this point certainly nothing of a net structure or even of a branching of single fibrils could be noticed, which, with Economo (15), we would here expect in a real net-structure. From the processes and from the interior of the cell the neurofibrils stream into the axone hillock, and they could be followed pretty far into the latter. All these findings speak no doubt against the existence of a neurofibril net-work in Mauthner's cell.

Ramón y Cajal (12) emphasized on the basis of his study by his own technic a reticular structure of the neurofibrils in the ganglion cells. He distinguished two kinds of fibrils—the primary and the secondary neurofibrils; described the existence of a superficial and a deep neurofibril net-work, and he stated that the parallel fibrils of the dendrites enter into correspondence with both the net-works. He declared, moreover, that the fibrils of the axis-cylinder originate from both these reticula. Retzius (26), v. Lenhossék, and Held (19, 20) produced by means of Cajal's method similar results as Cajal, while others (Marinscu, v. Gehuchten, and others cited in 15) maintained a certain reserve on this point, at least for some cell types. Bielschowsky (8), who studied the motor cells of the anterior horn by Cajal's method, pointed out the disadvantages of the latter, through which a false picture of a reticulum might be brought out. Wolff (28) expressed an opinion concerning the spatial relation between the honeycomb of the protoplasm and the neurofibrils and declared that the neurofibrils always lie in the wall of the former. According to Economo, the neurofibrils through simultaneous impregnation of this wall of the honeycomb pattern may look as if they were connected by cross-beams so as to form a net-work.

As far as the anastomotic connections between the neurofibrils are concerned, Wolff (28) did not want to speak of a real net-work; on the contrary, he assumed theoretically a plexus formation of the neurofibrils. Heidenhain also, on the basis of the theoretical considerations, assumed a metaplexus in the cell. In the present work, however, I will not enter further into theoretical considerations, but restrict myself merely to the microscopically visible things. I came to the conclusion that the neurofibrils in Mauthner's cell form no net-work at all. Above all I wish to emphasize here that I could not observe any neurofibril reticulum in this cell even in my best preparations with Cajal's and Levaditi's methods (fig. 2).

THE 'GOLGI NET' OF BETHE IN THE SYNAPSE OF MAUTHNER'S CELL

Careful investigation of the synapse of this giant cell by means of different methods revealed a most characteristic net-work, which covers the cell-body as well as its processes like a basket and also fills the 'axone cap' (figs. 3 and 4). There is no doubt that this net-work is identified with the structure, which was described for the first time by Golgi ('93) and later called the Golgi net by Bethe.

This net-work was constantly demonstrated in Mauthner's cell and was most beautiful in the Levaditi preparations: as far as I know this technic has never been applied before for the study of this structure. In Ameiurus brain the net-work appeared in a brown or dark color, whereas in Carassius it was stained in a brown to yellow color. As another advantage of this technic it must be emphasized that the nerve fibers in the synapse were also demonstrated as clean cut dark brown fibers simultaneously with the Golgi net, especially clear in Carassius. In the latter the Golgi net substance appeared mostly yellow and the contrast with the stain of the nerve fibers was so striking that it threw very good light on the problem of the mutual relation between both structures, which had been discussed by different authors and still had remained undecided. In both Ameiurus and Carassius the 'Füllnetz' of Bethe was also stained yellow in this method.

In the Heidenhain preparations the same structures were also demonstrated, especially clearly in formol-fixed material; but here the Golgi net could only be observed around the cell and in the 'axone cap.' On the surface of the Mauthner cell I could hardly see this structure, owing to the fact that the cell-body and the Golgi net appeared in too closely similar color (fig. 5). In the Heidenhain preparations (fig. 7) and the thionin-eosin preparations of formol-Zenker material one could hardly recognize the Golgi net-work, unless he has impressed the picture upon him in the above-mentioned preparations. In the Cajal preparations the Golgi net and the 'Füllnetz' were not observable.

I will first describe my findings in the Levaditi preparations. On the dendrites and on the part of cell-body which is not covered by the 'axone cap' the Golgi net is a single layer; that is to say, one layer of a net surrounds the cell and the dendrites. Each nodal point of this net-work is connected with the cell surface by means of a beam, which is attached to the cell surface perpendicularly, with a slightly extended basis (fig. 4). The 'axone cap' appears in a triangular shape in Ameiurus sections and is filled with more or less numerous layers of the Golgi net; the nodal points of the net layer, which lies closest to the cell surface, are connected with the latter by a Golgi net beam each. The meshes of the net-work are irregular and of variable size; but, generally speaking, the mesh is smaller the nearer we come to the cell. In Carassius the 'axone cap' has a round shape and here it shows a dense conglomeration of the Golgi net substance. I believe I can compare this heap of the Golgi net substance in the 'axone cap' with that conglomeration of the Golgi net structure which Bethe (7) found on the Purkinje cell of the cerebellum and in other parts of the central nervous system. Most meshes are five or six cornered, but there are many four-cornered and some seven- and eight-cornered ones. At the nodal point of the mesh-work three beams join together as a rule; the nodal points, at which four mesh beams unite together, are rarer. The nodal points of the mesh-work in both the 'axone cap' and on the cell surface are a little thickened, and in my preparations of Carassius brain some of them contrast clearly as deeply brown stained round points with the yellow or light brown impregnated Golgi net beams. These points might well be interpreted as the cross-sections of the nerve fibers in the synapse, as is to be described more precisely below. Unlike Bethe (7), who denied the direct connection between the Golgi net and the 'Füllnetz,' I could find the direct continuity of both the net-works, as has already been claimed by Held (18, 21), Economo (15), and others. The 'Füllnetz' pervades the whole gray and white matter and fills the space between the myelin sheaths, surrounding the latter with its net-work. At the nodal points and also in the beams of this mesh-

work we find in this preparation brown- or black-stained points of different caliber, which are certainly the cross-sections of nerve fibers. The 'Füllnetz' is less sharply marked, looks lighter than the Golgi net; the mesh itself is larger and more irregular and the mesh beams are thicker than those of the Golgi net-work. Contrary to the statement of Bethe (7), I could observe very distinctly that the Golgi net beams are connected with glia nuclei by means of somewhat extended bases, and also with the walls of capillaries.

In the Heidenhain preparations of formol material I could confirm similar relations of the Golgi net and the 'Füllnetz,' as described above (fig. 5). The nodal points of the net-work also were found a little thickened here and there. The only thing which is different is that the nervous elements here are stained in a color similar to that of the Golgi net, and the distinction between the nervous elements and the Golgi net becomes naturally difficult.

In both the above-mentioned preparations I observed indisputably the direct transition of neurites into the Golgi net beams, as is shown very clearly in figures 5, 6, and 11, although I do not mean by that at all that the Golgi net is of nervous nature. To this question of the nature of the Golgi net and its relation to the nerve fibers I will return later.

As already remarked, Held (18) demonstrated the net-like formation on the different kinds of the central ganglion cells, after Golgi had described it for the first time. Held at first identified his net-like formation with Golgi's net-work and characterized it as a dense net-work with coarse nodal points, formed by the fusion of arborized nerve fibers in the gray matter. He therefore described it as the 'pericellular nervous terminal net.' Besides Held, the net-like framework of the ganglion cell was also described by Semi Meyer (23, 24), Auerbach (2), and Bethe (7). Auerbach described the terminal nervous net around the ganglion cell on ground of his own method. Bethe, who demonstrated his Golgi net with the help of his molybden technic, concluded in harmony with Held, Semi Meyer, and Auerbach that

it is probably of nervous nature, because he believed to have found in the first place that the ends of the neurites are directly connected with the Golgi net and in the second place that the neurofibrils of the ganglion cells are combined with the nodal points of the Golgi net-work. But Held (18) raised the question whether the different authors have really seen the same structure. He said: "There is no doubt that certain net-structures of the Golgi preparations and the methylene-blue picture of Semi Meyer and Bethe's net-work are identical. What characterizes them is above all a certain monotony of the net figure and the stiffness of the net beams and further the nodal points of the mesh are generally not considerably thicker than the beams themselves." He then declared that Semi Meyer had seen something different, because Meyer's figures offered no proof of the nervous nature of his net-work, as it was stained isolated and without any relation to the nerve fibers. As regards Bethe's opinion, Held assumed that neither Golgi nor Bethe had furnished the necessary evidence. According to him, the statement of Golgi[3] that the neurofibrils of the gray matter unite with the net-work around the nerve cells proves nothing, because in Golgi's method the impregnation can jump from the branching nerve fibers to the thoroughly disconnected net structure and create a false connection between them. On account of the same reason, Held (18) denied the conclusiveness of his own Golgi preparations, in which the close relation between the nerve endings and the net-work was distinctly visible (fig. 8, table 13, 1902). Nor did the figures and the statement of Bethe about the transition of neurites into Golgi net beams seem trustworthy to him, although he was not able to give any special reasons for this. As far as the findings of Auerbach are concerned, Held thought that Auerbach had observed a similar structure and had interpreted it essentially alike. On the basis of these considerations, Held (18) came to the conclusion that on the surface of certain ganglion cells of vertebrates there exist two different kinds of net-

[3] Jahresbericht v. Merkel u. Bonnet, 1894. Cited in Held's 1902 (18).

work—the Golgi net and the nervous pericellular terminal net—which are quite distinct in their relation to other elements of gray substance and in their functions.

Held furnished as an argument for this hypothesis the fact that the nodal points of each net-work are distinct from each other. He figured round or star-shaped formations in the mesh of the Golgi net, connected among each other by delicate threads, which lay above or beneath the beams of Golgi net. He further claimed, pointing to the figures and statement of Bethe (7), that some round or oval specks, which are visible here and there as the contents of some meshes, might well be interpreted as the residue of discoloration of those star-shaped formations, which remained stained in his own preparations. Held (17) stated formerly that the surface of the ganglion cells are covered by the variably densely scattered and variably large protoplasm pieces, which have granular structure and are connected among each other with fine threads so as to form a net-work. At that time he spoke of them as the neurosome conglomerations on account of their thick granulations, and he identified them with the nodal points of his nervous terminal net, which he demonstrated by means of the Golgi method. Later he (18) identified these neurosome conglomerations with the contents of the Golgi net meshes referred to. Bethe (7) tried to argue that the neurosome conglomerations of Held must be regarded as the broken products of his Golgi net. Against this opinion, however, Held (18) furnished the proof that his neurosome conglomerations could be pretty clearly demonstrated in sections, which were prepared for Bethe's molybden method II by means of other stains, whereas in the alternating sections the intact Golgi net could be demonstrated by Bethe's stain.

In the following I wish to describe my results in Mauthner's cell in comparison with the findings of other authors in other cells. My observations led me to many interesting conclusions, which could not be brought into conformity with the findings of other authors without herein wanting to generalize those results in the fish brain immediately. In the first place, attention

must be called to the fact that in my Levaditi preparations and Heidenhain preparations of formalin material the meshes of the Golgi net were free from any formation or granule, which could be homologized with the neurosome conglomeration of Held. As the figures (6 and 11), produced from a Levaditi preparation of Carassius, show, the cell-body is covered by a net-work, whose nodal points are considerably thicker than the beams and appear as deeply brown-stained spheroidal points. This net-work passes directly into the net structure of the 'axone cap,' which is itself continuous with the 'Füllnetz' of Bethe. There can be no doubt that this net-work is the Golgi net. The meshes of this net-work are free from any further material, as the figures clearly indicate. In the axone cap, too, we cannot find any content in the mesh of the net-work. Above all, stress must be laid in these figures (6 and 11) upon the fact, that numerous nerve fibers, which have lost their myelin sheaths at the border of the 'axone cap,' run toward the surface of the cell, always keeping their place along the beams of the net-work. Moreover, we find the nodal points of the net-work here and there showing round brown and thick points, which could well be interpreted as the cross-sections of the nerve fibers, running along the beams of the net-work. Now and then we find a cross-section of a nerve fiber, which looks as though it lay isolated in the mesh of the Golgi net, but through careful observation with continual rotation of micrometer screw we realize that it is connected with the neighboring nodal points of the net-work by means of delicate yellow threads. On the surface of the Mauthner cell we also, find occasionally in the mesh of the Golgi net a certain granule, which might perhaps be homologized with the neurosome conglomeration of Held. This granule, which is seemingly an isolated round particle, but in reality a star-shaped structure with radiating beams, does not lie in the mesh of the net-work, but is found in a level different from that of the neighboring net beams, and with careful observation it is, at least at times, found to be connected with the Golgi net by means of the radiating beams.

In the Heidenhain preparations of formol material a similar

condition of the synapse was in evidence and the close relation between the nerve fibers and the Golgi net-work was very clear, as is shown in figure 5. In the Heidenhain preparations of formol-Zenker material the synapse appears denser than in the previous preparations in sections of the same thickness, but one can after some practice easily find the similarity of the net-work in the cell circumference and in the 'axone cap.' In figure 7, produced from this preparation, we observe here and there in the meshes of the Golgi net around the Mauthner cell a star-shaped granule which is found oftener here than in the previous preparations. It is obvious that these granules correspond to Held's neurosome conglomerations, which he located in the mesh of the Golgi net and interpreted as the nodal points of his 'peri-cellular terminal net.'

As was noted in previous preparations, these granules do not lie exactly in the mesh of the Golgi net, so far as my observations go. We cannot observe both these granules and the surrounding Golgi net beams with equal distinctness in one and the same focus of the microscope. By careful observation with slight rotation of the micrometer screw we could ascertain here as well that these granules are connected by means of their radiating threads with neighboring nodal points of the Golgi net. Although I noted also, as Held (18) did, that now and then the radiating threads do not pass over the neighboring nodal points, but reach the adjacent granules, passing either over or beneath the net beams, this should not be wondered at in the three-dimensional net-work. On the ground of these considerations, I am inclined to assume that these granules correspond to nodal points of the Golgi net, which lie in different levels and give the false idea that they are within the mesh of the Golgi net. Even in the thionin-eosin preparations a similar structure was recognizable, although it is much more difficult to perceive than in the previous preparations.

Held (18) figured the mutual relation between the neurosome conglomerations; but it is not indicated clearly enough in his figures (7 a, b, 11, 12, 1902). His Golgi figures (3, 4) showed the

situation more clearly; but his interpretation seems to be wrong in so far as he wanted to explain these figures as his 'nervous pericellular terminal net,' which according to his opinion lies alternating with the Golgi net on the surface of the nerve cell. I shall come back to the argument on this point later. As mentioned, Held (18) denied Bethe's opinion concerning the direct transition of the neurites into the Golgi net; also Economo (15) figured such a picture (table 3, fig. 25), but he remained in agreement with Held. I have already remarked that my preparations (figs. 5, 6, and 11) showed indisputably the close relation between the Golgi net and the neurites; I could even demonstrate pretty often delicate deep brown impregnated nerve fibers in the beams of the Golgi net, as shown in figures 3, 6, and 11, which arrive at the surface of the cell. Bethe (7) enumerated five points in his paper which according to him speak in favor of the connection of neurites with the Golgi net and in favor of the nervous nature of the Golgi net. With regard to his second argument, that the Golgi net is always dense and fine in places of the central nervous system where numerous axis-cylinders ramify, I would mention that in the axone cap of Mauthner's cell, where abundant axone-fibers form a dense plexus, the Golgi net structure offers a very thick conglomeration. As far as Bethe's third argument goes, concerning the probability of demonstrating the neurofibrils in Golgi net beams, I can explicitly assert that I could without doubt find the nerve fibers in the latter, as I stated already. I believe I have settled this point, on which Bethe (7) was not sure himself. At the same time I must emphasize that I could not confirm Bethe's statement, that the neurofibrils form a net-work in the net beams; as I shall discuss in the following chapter, I do not find any net-work of the nerve fibers in the synapse of Mauthner's cell. Although these arguments of Bethe, as Held (18) declared with good reasons, give merely indirect proofs for the nervous nature of the Golgi net, I think they indicate at least that the neurites have something to do with the Golgi net. I do not mean by that, however, at all, that the Golgi net is a nervous structure. Now one might

raise objection against me that the nerve fibers, which I have demonstrated in the beams of the Golgi net, might be neuroglia fibers. Concerning this I should raise the following points: in the first place, I could not, like Bartelmez (4), find any neuroglia fiber by means of specific neuroglia methods, and in the second place, I was able to follow the nerve fibers far back to the part where they have myelin sheaths.

As quoted above, the connection of nerve fibers with the Golgi net was stated by Golgi; also Held himself figured such points from his Golgi preparations (fig. 8, table 13, 1902). But he immediately denied the demonstrative power of these findings on account of the drawback of the Golgi technic mentioned above. I wonder why Held was so severe in his criticism concerning Golgi's method, when the picture was unfavorable to his opinion, whereas he avails himself of it to its full extent, if it is convenient for him. Held (17) formerly demonstrated (figs. 5 to 8, 1897) his 'pericellular nervous terminal net' around a certain kind of nerve cell. Later, however, he altered his interpretation of these figures so that only his figures 7 and 8 can hold their ground even in his extended critical consideration. If one, however, compares Held's figures 5 and 6 with 7 and 8, one can hardly see why in the figure 6 and in the light part of the figure 5, which showed the impregnation of the Golgi net, he should later have seen evidence of a nervous net-work. To me all these figures, especially 6 and 7, look very much alike; in the light part of the figure 5 the nodal points of the net-work are not thickened, but to my mind one should not be surprised at this in a method which is so inconstant in its results. Besides, it must be emphasized here that Held (17) did not rely on satisfactory evidence, when he set up his 'pericellular nervous terminal net.' He considered the pericellular net-work as the nervous-one merely for the reason that he found the connection between the net-work and the arborization of the nerve fibers and the thickening of the nodal points of the former. The relation between the Golgi net and the nerve fiber arborizations has been settled, I believe, through my present investigation. Moreover, despite the characteriza-

tion of the Golgi net by Held, I have found a thickening of the nodal points of the Golgi net, although I would not deny the fact that the nodal points sometimes, especially in my Levaditi's preparations of Ameiurus, were not particularly thickened, when the impregnation of the nervous elements was not sufficient. According to my opinion, however, this mark of distinction is not of considerable value and the variation must be regarded as the consequence of variable impregnation and methods of demonstration. It would be necessary to add here that in my Heidenhain preparations the nodal points appeared always thickened. On ground of these considerations, all the figures of Held seem to be pictures of the Golgi net, which is connected with nerve fibers and possesses the thickening on its nodal points; the latter might well be regarded as the cross-section of the nerve fiber or the so-called terminal foot of Held, which I will describe and discuss in the following chapter. The figures of Held's publication ('02; 3, 4) might come under the same point of view. Anybody who would compare those figures of Held with mine (figs. 6 and 11) would note immediately that they demonstrate thoroughly similar situations. I therefore came to the conclusion that on the surface of the Mauthner cell there exists a single net-work—the Golgi net—and that the endings of nerve fibers do not lie in the mesh of the latter, as it was supposed by Held, but the nervous elements of the synapse are related to the Golgi net and reach the cell surface at the same points where the Golgi net beams are attached to the cell surface. Held seemed to have been misled in his hypothesis on account of his one-sided interpretation of Golgi and neurosome preparations and his belief in the glious nature of the Golgi net.

As far as the nature of the Golgi net is concerned, the interpretation of Golgi,[4] Cajal (12), and Heidenhain (16), and Wolff (27), who wanted to see in it a neurokeratin structure, an artifact by coagulation, or then the superficial part of the honeycomb structure of the nerve cell, must immediately be denied.

[4] Cited in Plasma u. Zelle, Heidenhain, Bd. 1, a, p. 911.

Also the hypothesis of Bethe (7) who considered it as the nervous structure, I should deny positively with Held (18, 21), Economo (15), and others. Held discovered in both the white and gray matter of the brain a glia reticulum which provides myelin fibers with neuroglia sheaths and he identified this with the 'Füllnetz' of Bethe; he also demonstrated that the beams of the glia reticulum pass into those of the Golgi net, increasing thereby the density of its substance. Afterwards he added his further observation that the neuroglia cells accompanying the ganglion cells form the Golgi net of the latter. Economo found the direct connection between the Golgi net beams and the glia nucleus by pediform appendages. All these findings I could establish in the synapse of Mauthner's cell; there is no doubt now that the Golgi net is of glious nature; it must be added here that also Schiefferdecker,[5] Paladino (26), Besta,[6] and Alzheimer admitted its glious nature.

What, then, is the relation between the nervous elements and the Golgi net structure? As I remarked already, the myelin fibers are enclosed in glia sheaths of net-like structure, which is continuous with the diffuse glia reticulum. As far as I know, the relation of the unmedullated part of the nerve fibers to the glia tissue is not known very clearly. Held (19) said that, only occasionally and rather unevenly by means of Bethe's molybden technic, he obtained pictures in which the unmedullated fibers are enveloped in a delicate Golgi net. He added also that these sheaths of unmedullated nerve fibers are connected with the diffuse delicate glia reticulum by means of projecting spikes. This description of Held comes pretty near to my own findings in this work (nerve fibers in the beams of the Golgi net, cross-sections of nerve fibers at the nodal points of the net-work), which indicate that the unmedullated part of the nerve fiber is also enclosed in glia sheaths. I am very sorry that I could not establish the finer structure of these glia sheaths in the present

[5] Heidenhain, Plasma u. Zelle, Bd. 1, a, p. 911.
[6] Riv. di. patol. nerv. e ment., T. 16, p. 604.
[7] Nissl's Arbeiten, Bd. 3. 3, p. 412–421.

investigation: but on the basis of my findings I should be able to declare, I believe, that they accompany the neurites to their ends, connecting each other by means of delicate beams and thus forming the Golgi net in the axone cap as well as on the cell surface. Also the minute cap dendrites described by Bartelmez (4) are enclosed in the yellow net beams of the Golgi net.

Bielschowsky (18) also demonstrated the Golgi net by his method and he could even observe in harmony with Bethe (7) that nerve fibers stream on many points into the beams of the Golgi net. Further, he and Wolff (11) expressed themselves about the relation between Bethe's net-work and the 'terminal nervous net,' which they demonstrated as follows:

> The mesh-formation in our picture is not as regular as that which Bethe's technic yields. Also the net beams appear in ours more delicate than those in Bethe's. Still we consider it is probable that both methods demonstrate in this the identical structure. The difference might depend upon the fact that in Bethe's technic the plasmatic component of the net-work and in ours, the fibrillous component, becomes more manifest in the preparations. As Bethe (7) insisted, the beams of the net-work are not homogeneous but delicate fibrils are recognizable in them, which are continuous with the intracellular neurofibrils.

Now, if one compares this statement with my description, it becomes highly probable that the so-called 'plasmatic component' of the net-work of Bielschowsky and Wolff is to be interpreted as the Golgi net substance. As will be described in the following chapter, in some of my Bielschowsky preparations the sharp and intensely impregnated endings of neurites in the synapse of Mauthner's cell were seen connected with each other by pale stained beams forming a net-work. In the eosin counterstain of this preparation the sharp and dark stained components of the net-work were covered by more or less thick red sheaths and they were interconnected by red-tinged bridges so as to form a net-work (fig. 15). It was extremely interesting to find that the red-stained component of the net-work was directly continuous with the glia reticulum and with the glia nucleus. This picture is to some extent equaled by my findings in the Levaditi

preparations. Bielschowsky and Wolff (11), Held (20), and others took for granted that the transition of the axone-fibers into the nerve cell consists of axone-plasma and fibrils. I agree with this opinion. But I believe that the component of the net-work, which appeared red in eosin counterstain, would be the Golgi net substance, to some extent at least. The statement of Bielschowsky and Wolff (11) that the simultaneous existence of a glious pericellular net-work is compatible with their previous findings, can no longer be maintained, on ground of my above-described considerations.

THE NERVOUS TERMINAL FEET AND THE NERVOUS TERMINAL NET OF HELD AND THE NEUROFIBRIL CONTINUITY

The connection of neurones by means of special structures (nervous terminal feet) was first discovered by Held (17, 18), confirmed by Auerbach (2), and popularized by Ramón y Cajal (12, 13). Through the further investigations of many authors by means of Cajal's and Bielschowsky's methods many valuable contributions were added to this interesting and important problem. The question of the nervous pericellular terminal net and the neurofibril continuity also has long been the subject of dispute among the histologists. We are not as yet enlightened thoroughly on these questions.

I shall now go over these questions on the basis of my investigation on the Mauthner cell. In my Cajal preparations the nervous elements of the synapse were demonstrated almost exclusively as clean-cut deep brown fibers; the glia nuclei were the only things impregnated distinctly besides the nerve fibers. Figure 8 is reproduced from a preparation of Carassius; the lateral dendrite of Mauthner's cell is enveloped here in a sheaf of unmedullated nerve fibers. Each nerve fiber is provided with a thickening, which is to be homologized with 'bontones de Auerbach' of Cajal and lies more or less close to the surface of the dendrite. Besides this, some fibers have one or multiple thickenings on their way to the cell and sometimes present the picture of a string of pearls. There is no doubt, that these are iden-

tical with the 'bontones de trajecto' of Cajal. Both these 'bontones' are, generally speaking, spindle-shaped or spheroidal and variously large. As far as my observations reached, there is no particular difference in structure between these two kinds of 'bontones.' They are now and then impregnated massively or as if punched; most of them show, however, the splitting up of the axone fiber into several delicate neurofibrils in them, as figure 8 *x*, indicates very distinctly. Some of them appear in the preparations in cross-section and then they show the cross-sections of these delicate fibrils in them (fig. 8, *xx*). I must emphasize here that I did not find any net structure in the 'bontones,' as is described by many authors; the one, as we find in figure 8 at *xxx* looks as though it had net structure, but I believe it is by no means a real net-work; on the contrary, it shows merely the splitting up of the axone fiber into multiple delicate fibrils. Besides these large 'bontones' we find many minute rings, lying close to the surface of the dendrite, and these rings are continuous with very delicate fibers. The latter come from the other fibers as their ramifications or from the end of the large 'bontones' (fig. 8, *xxxx*). It is quite obvious that these rings are similar to those structures which were described and figured by Cajal (13). Now, the nerve fibers, which possess the above-mentioned qualifications, pass sometimes very near the surface of the dendrite, and thus remind us, especially in the tangential sections, of fibers coming into contact with the cell surface of the dendrite by means of their 'bontones de trajecto,' as was claimed by Cajal (13). But on careful observation, especially on examination of profile pictures, we can easily convince ourselves that there is no contact between them; at least I can state definitely that there are many of these 'bontones de trajecto,' which lie quite remote and free from the surface of the cell (fig. 8).

On the ventral dendrite of Mauthner's cell I found a similar condition in the synapse. In the 'axone cap' we see abundant unmedullated nerve fibers, which, as far as my investigation went, form a plexus of nerve fibers. Beccari (5) used the term 'canes-

tro,' to express the condition of nerve fibers in the synapse; but he did not give any statement about the behavior of the fibers with reference to each other. We find nerve fibers of multifarious directions and ramifications of the fibers are very often observed; but the anastomosis formation between them and even the net formation of the nerve fibers could not at all be observed in the 'axone cap.' The nerve fibers of the axone cap showed also the 'bontones' and the minute rings. The whole condition of the synapse is, generally speaking, similar to that of the lateral dendrite.

Cajal (13) stated and figured that the endings of nerve fibers come in contact with the cell surface not only by his 'bontones de Auerbach' and the minute rings, but also by means of the 'bontones de trajecto.' His figure 9 indicates that the latter lies quite close to the cell membrane of the ganglion cell. Economo (15) claimed to have observed that the axis-cylinder enters into connection with the nerve cell by multiple terminal feet, running on the surface of the ganglion cell. Heidenhain (16) declared further that most of the nerve cells carry a very thick pelt of end-knobs. As already described, there was no evidence in my preparations of the connection between the cell surface and the 'bontones de trajecto.' At least I can state that I have found many of these 'bontones' quite free and remote from the cell surface. The figures of Cajal (13) and Economo (15) do not prove that they are connected with the latter. I have the idea that they present merely an optical illusion, caused by the nerve fibers with those 'bontones' passing quite near the nerve cell.

Cajal (12) at first characterized his 'bontones' as spheroidal or elongated structures, impregnated solidly or at the most punched. He regarded them as the foundation of his contact theory, as he believed to have found that the nerve fibers end on the cell surface with those structures. Dogiel (14) also described ring- or net-shaped terminal structures and remained a partisan of the contact theory. According to Held's (19, 20) observation with Cajal's method, the neurofibrils of his terminal feet form a net-work and communicate directly with the neuro-

fibril net-work of the nerve cell in two different ways. In one group of the terminal feet the net-work is connected with the intracellular neurofibrils by means of a feebly stained neurofibril net-work, embedded in a homogeneous substance, and in the other group delicate neurofibrils enter the cell body from the terminal feet in a radial direction, also surrounded by homogeneous material. Holmgren (22) distinguished also two types of terminal feet; his first type was of annular shape and his second type showed a tiny net-work, which is connected directly with the neurofibril net of the nerve cell. Later Cajal (13) also figured and described net-shaped terminal and transitional knobs and minute ring-shaped structures, which he believed to come into contact with the cell surface. Heidenhain (11) declared even that these are nothing but the net corpuscles, which appear in the peripheral nerve endings. In my Cajal preparations, however, the net-like structure of these nerve endings, as remarked, did never come to my observation. Some of the terminal feet looked merely granular in my preparations, owing to a fine silver precipitate; occasionally I observed in them a false net figure caused by the irregular distribution of the latter. But the pictures which are shown in figure 8 exclude beyond doubt the net figure of the 'bontones.' I wonder if the terminal feet possess really the net structure, as it was claimed by Held (19, 20), Holmgren (22), Cajal (13), and others. I suppose that this net figure comes from artificial admixtures of the impregnation of the honeycomb structure of the neuroplasm, which occurs now and then in Cajal's technic. To this question I shall return later.

As far as my observations go, the terminal feet must not be regarded as the definite ends of the axone fibers, as was assumed by Cajal and others. In my Cajal preparations I observed very often that single or multiple delicate neurofibrils come from the end of the terminal feet and advance toward the cell surface, embedded in a homogeneous substance and entering the cell body (fig. 12). I am very sorry to confess, however, that I could not demonstrate definitely the neurofibril continuity in

these preparations, as the intracellular neurofibrils were often not impregnated quite distinctly in them. So my finding corresponds to that type of the terminal feet of Held, in which the fibrils were followed in radial direction from the terminal feet into the cell body; the connection by means of a fibril net-work did not come to my observation.

Figure 9 demonstrates the condition of the synapse in the Bielschowsky preparation; on the surface of the axone cap, we see a number of nerve fibers going into the region of the 'axone cap;' Weigert and Heidenhain preparations show that they lose their myelin sheaths just at the border of the 'axone cap.' Some of these nerve fibers have each a large club-like expansion here and before the loss of their myelin sheaths. According to my experience, this phenomenon is more noticeable in Ameiurus than in Carassius; in regard to the significance of this finding I am not able to say anything definite as yet. On their way to the surface of the cell the unmedullated fibers have single or multiple spheroidal or spindle-shaped swellings; sometimes the fibers look like a string of pearls showing many of these swellings. There is no doubt that these enlargements are identical to the 'bontones de Auerbach' and the 'bontones de trajecto' of Cajal. In my preparations these nodes came out partially diffusely black and partially punched as Bielschowsky (18) described them. Besides these I could find also 'bontones' with splitting of the nerve fibers into numerous delicate fibrils (fig. 13), just like those which were described above in my Cajal preparations. The net-work, as was described by Wolff (27) from his Bielschowsky preparations, I could not demonstrate at all in them. In my Bielschowsky preparations I could not find any place, where the contact between the 'bontones de traecto' and the cell surface takes place, as was described and figured by Cajal. It is quite obvious in my figure 9 that at least there are many of those 'bontones' which lie quite remote and free from the cell surface. From the peripheral end of the terminal feet single or multiple delicate fibrils come out and proceed toward the cell surface, surrounded by the homogeneous substance, and enter the cell

body communicating directly with the intracellular neurofibrils. Figure 10 demonstrates clearly that the neurofibrils from the terminal feet enter the cell body. In this figure it is also shown that the intra- and extra-cellular neurofibrils communicate with each other; one might raise the objection against me, that the fibers which enter the cell-body might be the 'cap dendrites' of Bartelmez (4). On this point I can give the following arguments: first, some of the fibers could be traced back to the part, where they are to be enveloped in myelin sheaths, and, second, it is connected with the cell by means of the terminal foot (fig. 10). Terminal ramification of nerve fibers occurs very often within the 'axone cap;' between the ends of individual nerve fibers neither anastomosis formation nor even a simple net formation of nerve fibers came to my observation in the 'axone cap' oron the cell surface. Through careful examination I could always isolate the sharply and intensely dark stained nerve fibers from each other.

In the previous chapter I already stated that through the simultaneous impregnation of the Golgi net substance a false picture of a nervous net-work becomes observable in the synapse. According to my experience, the Golgi net substance is to a certain extent antagonistic in its staining reaction to the nerve elements in the Bielschowsky method; when the nervous elements are brought out sharply and dark enough the Golgi net substance remains unstained or is stained quite feebly, whereas the latter is impregnated more or less intensely when the former is stained more feebly. I also stated above that by means of the eosin counterstain the Golgi net substance is demonstrable in red color and is connected directly with the glia reticulum and glia nucleus on the one hand and the nervous elements on the other hand. Figure 15 shows that the nervous elements are covered with a more or less thick layer of the red-stained substance; also the terminal feet are surrounded by the same substance. This picture corresponds to that of the Levaditi preparation, which was described in the previous chapter.

After this description of my results in both the Cajal and Bielschowsky preparations I go over the discussion of the ques-

tions referred to. As remarked, my findings in the Cajal and Bielschowsky preparations are in opposition to those of Held (19, 20), Cajal (13), Holmgren (22), Wolff (27), and others, in so far as I demonstrated in the terminal feet merely the splitting of the nerve fiber into fine fibrils instead of a net structure. Now it is remarkable that some of the above-mentioned authors (Cajal, Held, and others) assume also a net structure of the intracellular neurofibril, while others (Wolff, 27) deny the net structure in the cell body, at least in many kinds of nerve cells. I could not find any real net structure in either the Mauthner cell or in the terminal feet. It appears extremely interesting to me that in Wolff's (27) figures Bütschli's honeycomb structure was stained in the cell body as well as in the dendrites. Economo (15) supposed that the net figure of the terminal feet might depend upon the simultaneous impregnation of the Bütschli structure. Also Auerbach (2), who was at first a partisan of the contact theory, declared that the neurofibrils do not form a reticulum in the terminal feet, but that one, two, or three delicate fibrils go radially into the cell body, embedded in the ground substance of the terminal feet.

Ramón y Cajal (12, 13), Dogiel (14), Retzius (27), Heidenhain (16), and others despite the repeated argumentation of the antagonists Held (19, 10), Holmgren (22), Bielschowsky (9), Antoni (17), Auerbach (3), and others) remained firm on the standpoint of the contact theory. According to Held, Bielschowsky, and others, however, the theory of the contact must result from the imperfect impregnation of the structure in question; they observed, as remarked, that the fibrils of the terminal feet go into the cell body and enter into relation with the cell fibrils. I could also confirm the neurofibril continuity in Mauthner's cell in the Bielschowsky preparations. The 'bontones de Auerbach' of Cajal evidently must not be regarded as the contact organs, in which the nerve fibers come to their ends, but are rather to be interpreted as the stations in the course of nerve fibers, where the modification of the substance takes place, which was claimed by Bielschowsky (8). With the supposition of the latter, how-

ever, who took for granted that here the dissolution of the nerve fibers occurs as a consequence of the loss of the perifibrillar cement substance, I cannot agree. On the contrary, I assume that the dissolution of the fibers is to be regarded as the result of the accumulation of the perifibrillar neuroplasm.

Bartelmez (4) described in the 'axone cap' of Mauthner's cell two kinds of endings—'free endings' and 'knob endings,' which latter are in contact with the cell surface; besides these he mentioned on the lateral dendrite 'club endings.' As far as my investigation went, the 'free endings' of Bartelmez are very hard to accept; though I found in my preparations nerve fibers which do not reach to the cell surface, it is always probable that they appeared in section. Nor can I find any essential difference between the endings in the 'axone cap' and in the lateral dendrite, as already described. Moreover, he did not state the structure of these endings in detail; he figured as 'pericellular net' (figs. 12, 13) a minute solid or ring-shaped structure, which is perhaps identical with the ring-shaped ending apparatus described by Cajal. Above all I must emphasize that I found the neurofibril continuity on the cell surface as well as on the dendrites; the 'plasma membrane,' which Bartelmez described on the surface of his club endings, must not be regarded as the last end of the nerve fibers.

Held (18) assumed a 'pericellular nervous terminal net,' which exists on the cell surface alternating with the Golgi net, as remarked before. In the previous chapter I showed that there is only one net-work on the cell surface formed by both the Golgi net and the nervous elements. Now, in my Cajal preparations, which do not show the Golgi net substance, I was not able to find any net-work in the synapse. I showed that Held's figures of his neurosome and Golgi preparations do not argue for his hypothesis. It appears extremely interesting to me that in his Cajal preparations the deeply stained terminal feet are not at all (19) connected by bridges or at the most connected among each other by feebly (20) stained bridges. Moreover, it must be remembered here that in the neurosome preparations of Held

the 'terminal feet'—the nodal points of his terminal net—were chiefly granular, whereas the beams between them appeared free from granules. Auerbach (2, 3) also assumed the existence of the nervous terminal net, whose nodal points coincide with his 'terminal knobs;' but his figures prove nothing. Besides, it must be emphasized that his net-work possesses a three-dimensional character not only on the cell surface, but also in certain other parts of the central nervous system—the substantia gelatinosa and the molecular layer of the cerebellum. The existence of a nervous net-work of this sort, which would penetrate the gray matter of the central nervous system, was denied by many authors (Cajal, (13) Kölliker (cited in 9), Retzius (26), etc.). Even Held (20) admitted that by means of Cajal's technic he could observe merely the indication of the extension of the 'pericellular nervous terminal net.' The figures of Holmgren (22) also prove nothing.

Concerning the pericellular net-work, which is found now and then in Bielschowsky preparations, I expressed my idea before, that there might be a false picture caused by the simultaneous impregnation of the Golgi net. This consideration will perhaps be strengthened by the description and the figures of Wolff (27). He figured on the surface of the cells as well as between the latter a net structure, which shows a striking resemblance to Bethe's net-work and is mainly stained more feebly than the terminal feet, which latter are directly continuous with the former. In the text he stated that the axone fibrils are not naked, but are enveloped in a mantle of bubble-like structure, which goes over into the honeycomb structure of the cell border. On the ground of his findings and Gegenbauer's intercellular bridge theory, Wolff declared that Bethe's pericellular net-work is nothing but the impregnated honeycomb structures of the cell border, the neuroplasmatic anastomoses and the perifibrillary mantles. That Bethe's net-work is not, however, to be regarded as the honeycomb wall, but as a glious structure, I have already stated.

SUMMARY

1. In the 'axone cap' and on the surface of Mauthner's cell a Golgi net structure was very distinctly demonstrated by means of Levaditi's method; in Heidenhain and other preparations a similar net-work was brought out. The Golgi net is of glious nature and is in close relation to the nervous elements in the synapse. According to the results of this study, the unmedullated parts of the nerve fibers are also enveloped in a sheath of glious tissue; the finer structure of this glia sheath is as yet unknown.

2. The hypothesis of Held concerning the existence of two kinds of net-work on the cell surface—a Golgi net and a 'pericellular nervous terminal net'—is denied; there exists, as far as my observations go, only one net structure, which is formed by both the nervous and the glious tissues. The so-called pericellular nervous terminal net is not to be regarded as a real nervous net-work, but to be considered as a picture produced by the simultaneous stain of the Golgi net-work.

3. The contact theory is a histological impossibility. The terminal feet cannot be regarded as the specific contact organs, but as the points in the course of axone fibers, where the dissolution of fibers takes place. The continuity of the intra- and extracellular neurofibrils is very clearly demonstrated.

4. The intracellular neurofibrils do not form any reticulum in Mauthner's cell; nor did the net structure which was described by many authors in other animals, ever come to my observation in the nervous terminal feet. The nerve fibers showed merely a splitting into numerous delicate fibrils in them.

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All figures are taken from preparations of Mauthner's cell.

The photomicrographs were not retouched at all. An achromatic Zeiss ocular no. 4 and a Zeiss oil-immersion $\frac{1}{12}$ were used. In figures 1 and 8 the length of bellows was 100 cm. and in all the others it was 60 cm. The figures 11, 12, 13, 14, and 15 were drawn using the Abbe camera lucida with slight rotation of the micrometer screw. (Apochromatic Zeiss ocular no. 4, oil-immersion $\frac{1}{12}$, tube length 20 cm.) In those drawings except 12 and 13 the glia structure is presented in a gray, and the nervous structure in a dark color. It is to be added here that figure 7 came from a fatigue preparation.

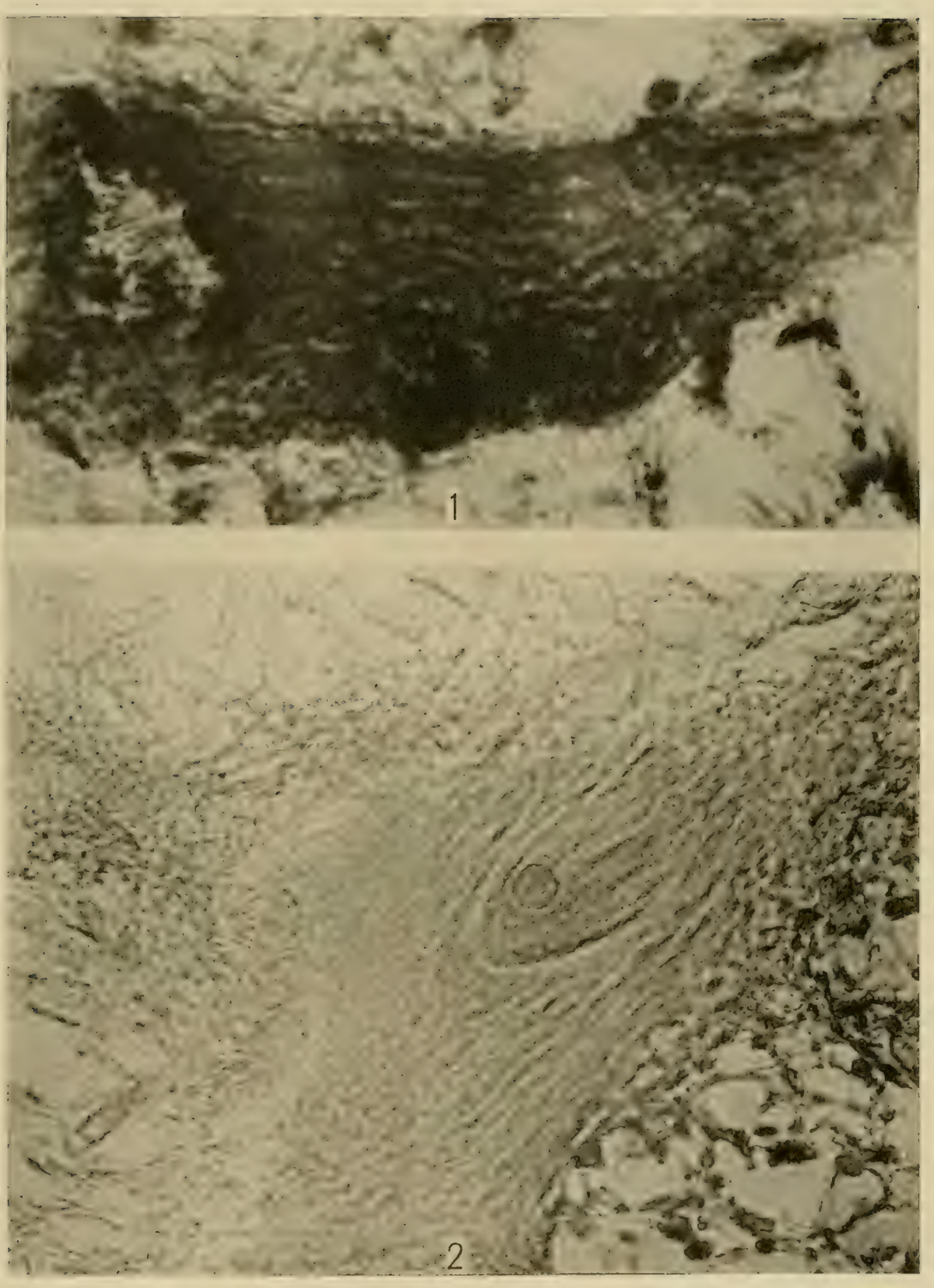
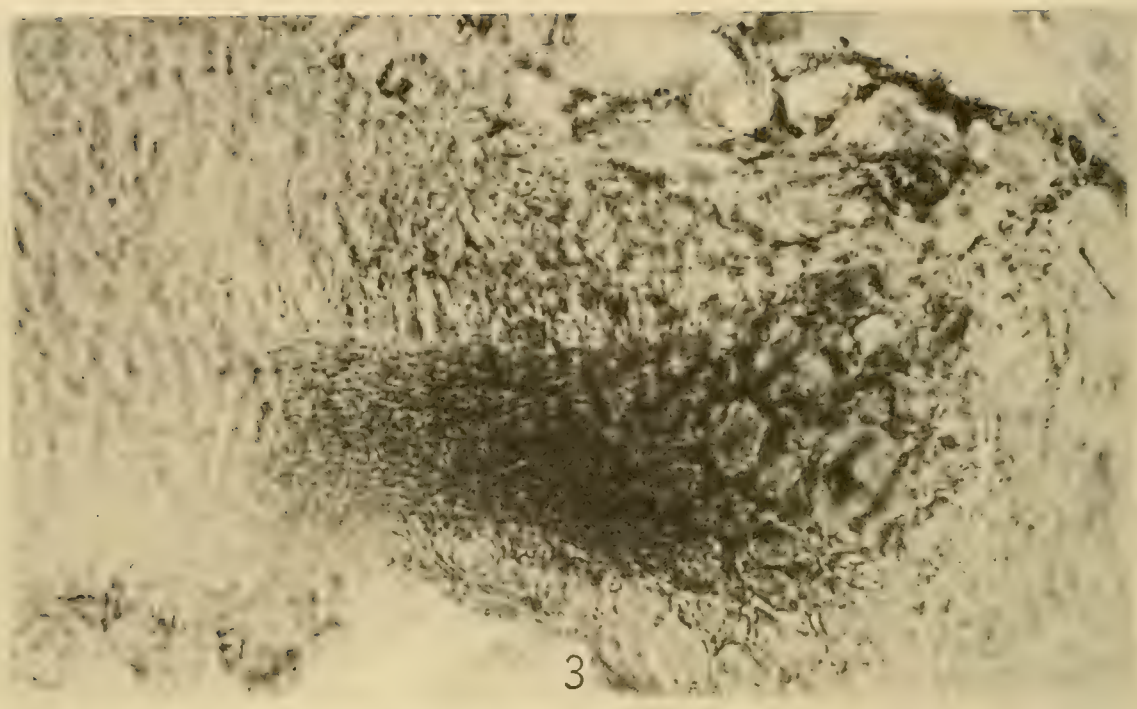


Fig. 1 Carassius auratus. Bielschowsky preparation.
Fig. 2 Carassius auratus. Levaditi preparation.



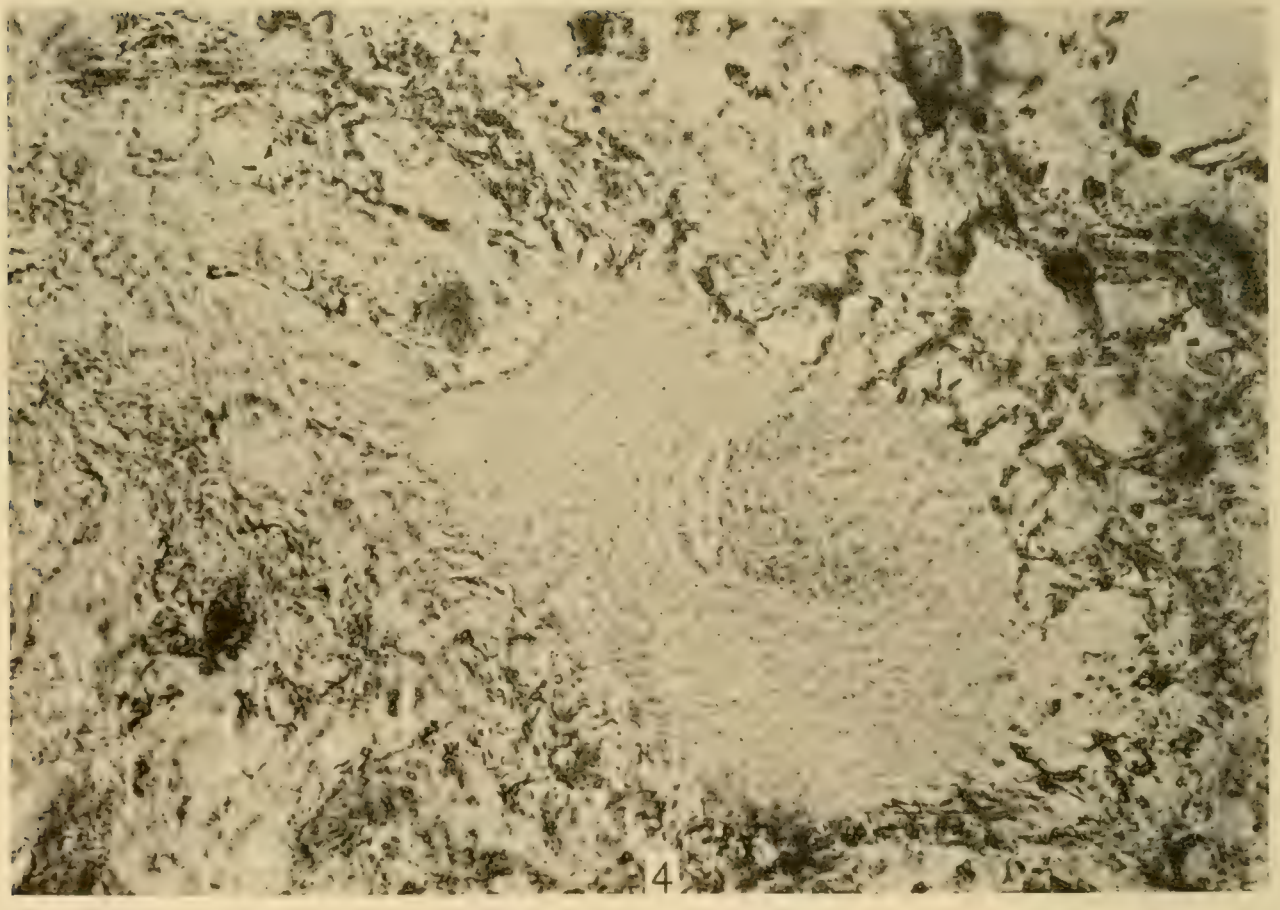


Fig. 3 Ameiurus nebulosus. Levaditi preparation.
Fig. 4 Ameiurus nebulosus. Levaditi preparation.



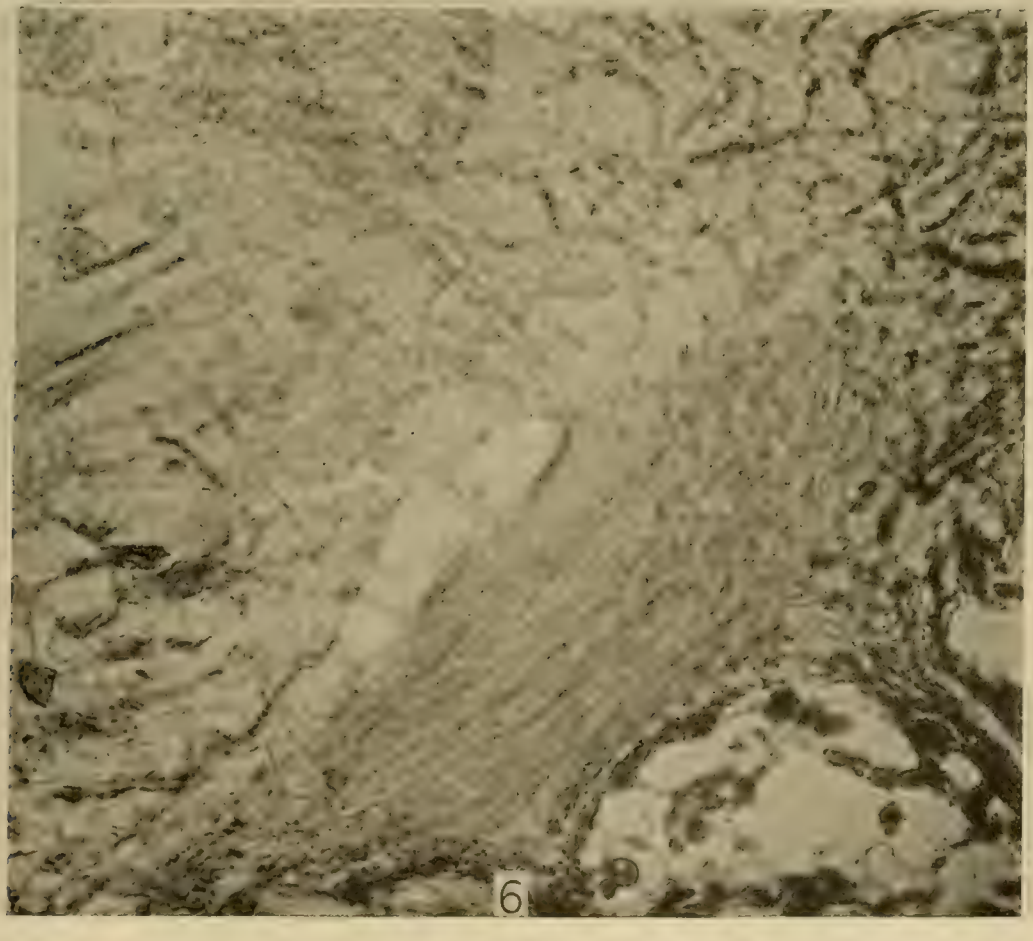


Fig. 5 Ameiurus nebulosus. Heidenhain preparation (formol material).
Fig. 6 Carassius auratus. Levaditi preparation.





Fig. 7 Ameiurus nebulosus. Heidenhain preparation (formol-Zenker material).

Fig. 8 Carassius auratus. Cajal preparation.





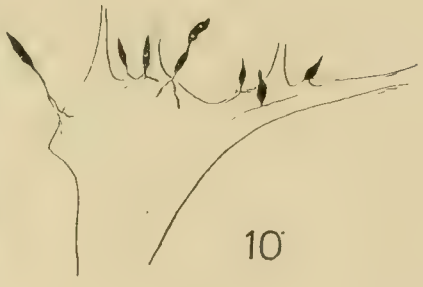


Fig. 9 Ameiurus nebulosus. Bielschowsky preparation.
Fig. 10 Ameiurus nebulosus. Bielschowsky preparation.

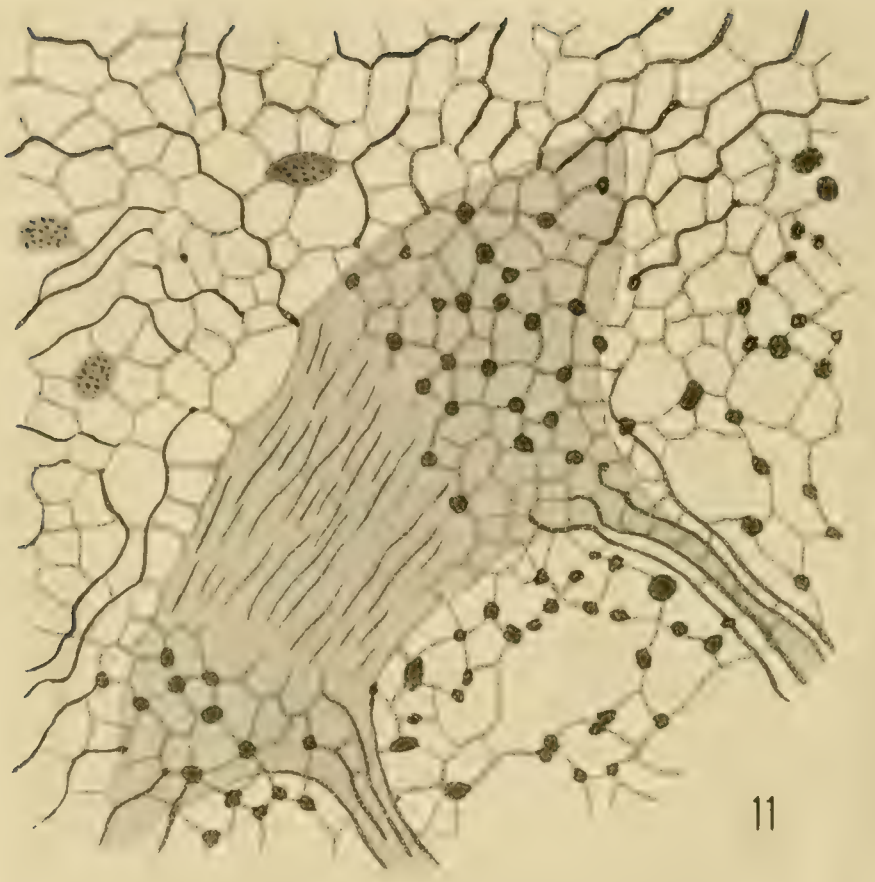


Fig. 11 Carassius auratus. Levaditi preparation.
Fig. 12 Carassius auratus. Cajal preparation.
Fig. 13 Ameiurus nebulosus. Bielschowsky preparation.



14



15

Fig. 14 Carassius auratus. Levaditi preparation.
Fig. 15 Carassius auratus. Bielschowsky preparation with eosin counter-stain.



C. Golgi

AUTHOR'S ABSTRACT OF THIS PAPER
ISSUED BY THE BIBLIOGRAPHIC SERVICE

APPLICATION OF THE MARCHI METHOD TO THE STUDY OF THE RADIX MESENCEPHALICA TRIGEMINI IN THE GUINEA-PIG

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THIRTY-FIVE FIGURES

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INTRODUCTORY

A little over a year ago I attended an autopsy of a little girl who died from a large glioma which involved most of the right side and a large part of the left side of the pons. Dr. Lawrence Selling had charge of the case and gave a very complete and accurate history. It seems that the growth of this tumor was most rapid, not more than six weeks, and a much shorter time was consumed since the trigeminal nerve was affected; so that secondary degeneration could not have been a factor in the disintegration of the mesencephalic root. The tumor

was more or less spherical in shape, extending from in front of the inferior olive to the nucleus of the oculomotor nerve. Serial sections of the brain, after a modified Marchi treatment,[1] demonstrated that the center of the tumor was in the neighborhood of a section through the trigeminal motor nucleus. Here the tumor had pushed up a narrow strip of the brain substance into the fourth ventricle. So far as could be determined, it had not severed the mesencephalic root fibers, but had injured all of the trigeminal root fibers on each side, in their course through the brachium pontis. In the region of the trochlear nucleus, or a little caudal (fig. 1), the tumor still occupies a large part of the pons and had pushed a narrow strip of the brain substance up into the aquaeductus cerebri (Sylvii), but apparently had not destroyed the trochlear nucleus nor the cells and fibers of the mesencephalic roots. Since both of the trochlear roots show marked degeneration throughout, it is highly probable that the trochlear nucleus was affected by pressure from the growth of this tumor.

A glance at the mesencephalic root almost anywhere in its course in the brain stem (figs. 1 and 2, *Mes.V.*) shows a very complete degeneration of its fibers. Inasmuch as the direct injury of its fibers occurred in the region of the brachium pontis, it would appear that the cells of origin were for the most part located in the semilunar ganglion and that the mesencephalic root fibers were mainly ascending. To test the truth of this assumption I severed the left trigeminal roots of several guinea-pigs immediately behind the semilunar ganglion and traced the mesencephalic root centrally to the midbrain. From these sections it is clear that the mesencephalic root contains ascending fibers, but decidedly insufficient to account for the marked degeneration of this tract in the series of the little girl. From still later experiments, where the mesencephalic root in a

[1] Material was thoroughly fixed in 10 per cent formalin and washed in running water. Pieces 5 to 10 mm. thick were placed for a week in a fluid of the following proportions: Osmic acid, 1 gram; iodide of sodium (NaI_3), 3 grams; water, 300 cc. It was finally washed in running water for twenty-four hours, dehydrated thoroughly, and embedded in collodion.

number of guinea-pigs had been severed, a little behind the inferior colliculus (corpora quadrigemina), and the degenerated fibers were traced into the motor root and nerves, I became convinced that by far the greater number of fibers in the mesencephalic root were descending rather than ascending, and that the mesencephalic nucleus or root in the little girl must have been injured by pressure in the region of the midbrain, if not through direct destruction by the tumor. The details and discussion of the results of the above-mentioned experiments will form the basis for this paper.

REVIEW OF THE LITERATURE

Johnston and Edinger have given a very complete review of the literature of the mesencephalic root to 1909 and 1911, respectively, so that it is inadvisable to go into detail over the early history of this work here. It seems that the first investigator of this root, namely, Meynert, considered it as sensory, but the majority of the authors, Forel, Koelliker, Van Gehuchten, and Cajal, regarded it as motor. Several of them, however, called attention to the similarity of the balloon-shaped unipolar cells of the mesencephalic root to spinal ganglion cells. From Golgi preparations Cajal noted that many very fine collaterals were given off from the mesencephalic root fibers to form a delicate network about the bodies of the trigeminal motor cells. He suggested that a relatively very weak stimulus could be intensified into a very strong one by such a mechanism. According to Cajal, the locus coeruleus cells constitute a separate system, which is not directly connected with the trigeminal nerve. On the other hand, Held claims that the mesencephalic root takes origin from cells in the mesencephalon and from the locus coeruleus. Bregmann states that a lesion of the motor portion of the trigeminal nerve produced a degeneration of the mesencephalic root. Wallenberg obtained a descending degeneration of the mesencephalic root as a result of a lesion of the tectum mesencephalic in birds.

Johnston made a most comprehensive morphological study of the mesencephalic root from Weigert serial section of the brains

of several fish, Amphibia, Mammalia, and a 15.5-mm. human embryo. In addition, he studied the cells of origin of this root in the mesencephalon from Cajal and Bielschowsky preparations. The author found these globular cells to be mainly unipolar, though some were bipolar or even multipolar; the bipolar cells were said to be more common in the lower vertebrates and in embryos. He also called attention to the resemblance of these cells to spinal ganglion cells, and regarded the large descending process of one of these cells as comparable to the peripheral process or dendrite of a spinal ganglion cell, and the more slender process of a bipolar cell he took to be the axone. In adult mammals Johnston suggests that the true axones may have been lost and the collaterals, described by Cajal as going from the peripheral processes to form networks about the trigeminal motor cells, may function as axones. From his Weigert series Johnston traced the mesencephalic root fibers into the sensory root of the trigeminal nerve. He was the first investigator to call attention to the fact that the mesencephalic root cells in the midbrain lie in the alar rather than in the basal plate and he is of the opinion that they represent neural crest cells which were not extruded when the medullary folds of the midbrain rolled up to form a tube. The author's conclusion is that the mesencephalic root is sensory rather than motor.

So far as can be learned, May and Horsley have given us the first important experimental work on the mesencephalic root. They experimented on cats and monkeys and studied several clinical cases, approaching the problem from the standpoint of chromatolysis and Marchi staining of medullary sheath degenerations.

As a result of a lesion of the mesencephalic root at various intervals in different animals from its passage through the inferior colliculus to a region immediately proximal to the gasserian ganglion, together with severing the ophthalmic, maxillary, the motor portion of the mandibular, and the mandibular nerves in other animals, the authors found decided chromatolytic changes in the globular cells of the mesencephalon in every instance, except the experiments in which the ophthalmic and maxillary divisions

of the trigeminus were cut. They call attention to the fact that these observations do not support Johnston's view that the axones of the mesencephalic root enter the trigeminal sensory root. Furthermore, they found that no chromatolysis took place in the locus coeruleus cells, indicating that these cells do not belong to the mesencephalic root.

In order to determine the ultimate distribution of the centrifugal (descending) axones of the mesencephalic root, May and Horsley applied the Marchi method of staining the degenerated medullary sheaths, after first destroying this root in the region of the inferior colliculus. They made serial sections of the brains and gasserian ganglions and also sectioned identified portions of the main trunks of the trigeminal nerves peripheral to the ganglion, from which they were able to trace the mesencephalic root fibers around the trigeminal motor nucleus and out the motor root of the trigeminal nerve to the gasserian ganglion. Since no degenerated fibers were found in the nerve trunks peripheral to the ganglion (ophthalmic, maxillary, and mandibular nerves), they thought that these fibers ended in the gasserian ganglion. The authors observed that this mode of termination of the mesencephalic root fibers would seem to be incompatible with their previous statement that some chromatolysis occurs after the mandibular nerve had been cut, but call attention to a point made by Warrington that chromatolytic changes resulting from a lesion of the anterior and posterior roots of a spinal nerve affect not only the cells in direct association with the fibers divided, but also other cells forming a part of the same nerve center. So that they appear to discount somewhat their first results obtained from a study of chromatolysis.

In a clinical case where the gasserian ganglion had been destroyed and the patient lived several weeks after the operation, May and Horsley found from an examination of Marchi sections of the brain stem that there were many fine and a few coarse degenerated centripetal (ascending) fibers in the mesencephalic root. In some experiments on cats and monkeys where the trigeminal nerve fibers were severed centrally and peripherally

to the gasserian ganglion, degenerated fibers were found in the former, but not in the latter.

According to these authors, the animals in which the mesencephalic root had been severed showed no loss of power of the masticator muscles or any functional loss of any kind.

From these experiments May and Horsley concluded that the mesencephalic root contained both centrifugal and centripetal fibers. The latter are sensory arising mainly from the gasserian ganglion, while the former take origin from the globular cells of the mesencephalon, but the authors do not explicitly state whether motor or sensory. They found, however, from Marchi preparations, after a lesion had been made in the inferior colliculus, that degenerated mesencephalic root fibers could be traced through the motor root to end apparently in the gasserian ganglion.

In 1911 Willems published a most comprehensive monograph on the mesencephalic root and masticator nucleus of the rabbit. His investigations were based on anatomical-histological and experimental methods. In the former he noted the difference between the globular unipolar cells of the mesencephalic root nucleus and the multipolar motor cells of the masticator nucleus in a 22 mm. and adult brains; while in the latter he counted the number of chromatolytic cells in the mesencephalic root nucleus after one of the motor branches of the trigeminal nerve and in one experiment the ophthalmic and maxillary nerves were cut. The experimented animals were killed ten to eighteen days after operation and sections of these brains were stained with toluidine blue or neutral red. In addition to giving complete descriptions of his experiments, the writer has summarized his results in tabular form. He found a disintegration of many cells in the mesencephalic root nucleus as the result of severing the masseter, sphenoidal, or temporal nerves, and a lesser number after cutting the external or internal pterygoid nerves, while no degenerated cells or scarcely any appeared after a lesion of the digastric or mylohyoid nerves. The writer cites a number of reasons for establishing the mesencephalic root as a sensory nerve for the masticator muscles. It will be seen that Willems'

results, while decidedly more extensive, confirm in the main the chromatolytic studies of May and Horsley.

A year later Kosaka approached this problem from the standpoint of chromatolysis. He experimented mainly on dogs, but made use of rabbits and monkeys. His method of procedure was to tear out one of the trigeminal nerve branches or muscles innervated by one of these nerves and count the number of degenerated cells in the mesencephalic root nucleus on the operated side. The author found if the third division of the trigeminal (N. mandibularis) was destroyed in the rabbit, dog, and monkey that he obtained a complete chromatolysis of nearly all of the cells of the mesencephalic root nucleus on the side of the operation, while no chromatolysis resulted from injury to the first division of the trigeminus (N. ophthalmicus). Upon severing the second division of the trigeminus (N. maxillaris) in the dog and monkey, he found a few degenerated cells in the mesencephalic root nucleus, the number varying according to the region of the lesion. In four experiments on dogs 12 to 59 disintegrated cells were found in a nucleus comprised of 1116 to 2427 cells and in a monkey 71 disintegrated cells appeared in a nucleus comprised of 2744 cells. No chromatolysis in the rabbit as a result of cutting the N. maxillaris. Furthermore, Kosaka reports that a lesion of almost any branch, sensory or motor, of the N. mandibularis brought about a degeneration of some of the cells of the mesencephalic root nucleus on the side of the injury. For example, in the N. lingualis 7 cells underwent chromatolytic changes and in the case of the digastric nerve 7 cells disintegrated. When the M. tensor veli palatini were removed from one side 22 cells degenerated, in the case of the M. temporalis, 30, and in the M. masseter, 20. It will be seen in connection with the last two nerves that Kosaka found considerably less chromatolysis in the mesencephalic nucleus than Willems and less than one would expect to be present.

Kosaka concluded that the mesencephalic root cells are concerned with both muscle and cutaneous sensations, and that they are more or less localized in the midbrain, the muscle sense cells being situated below the trochlear nucleus. He agrees with

May and Horsley that the locus coeruleus cells have no connection with the mesencephalic root.

I have been unable to obtain copies of any of van Valkenberg's papers and consequently know nothing of their contents.

In fact, my own studies of the mesencephalic root were completed or nearly completed before I received copies of the three previous papers, so that my results were obtained entirely independent of their investigations.

ASCENDING SENSORY FIBERS IN THE MESENCEPHALIC ROOT

1. Experiments

In order to establish whether there were any ascending sensory fibers in the mesencephalic root taking origin from cells in the semilunar ganglion, the left trigeminal roots were severed immediately behind the semilunar ganglion in a number of guinea-pigs. After allowing the animals to live for fifteen days, the brains with the trigeminal roots intact were removed and treated according to Simpson's modification of the Marchi method for demonstrating degenerated medullary sheaths, and were sectioned and mounted serially. The reasons for selecting the guinea-pig for this experiment rather than some larger animal were as follows: 1) The comparative ease with which the trigeminal roots can be reached. It is not necessary to drill through the skull in the guinea-pig to sever the trigeminal roots, because the semilunar ganglion is situated immediately above a large foramen (fig. 4, *S.F.*) in the alisphenoid bone. 2) The brain stem, which has essentially the same functional arrangement as the dog and cat, is much smaller, permitting of a thorough penetration of osmic acid through a piece extending from behind the entrance of the trigeminal roots to the cephalic end of the superior colliculus (corpora quadrigemina), and in addition requiring the handling of fewer sections to complete a series.

Operations. All operations were performed under sterile conditions, using ether for the anesthetic. The animal was placed on its back, a longitudinal incision was made through the skin

directly median to the mandible. The margins of the cut skin were clamped on either side to a sterile towel and the skin was separated from the fascia below. With the aid of a probe the muscles and blood-vessels of the mandibular region were separated until the digastric muscle was reached, which was severed through its tendinous portion and its posterior belly removed. This brings the tympanic bulla (fig. 4, *T.*) into view. In the first experiments the cephalic portion of the ventral wall of the bulla was removed with the aid of a small pair of bone forceps, exposing the inner ear. This incision was continued cephalad and dorsad to include the removal of enough of the caudal rim of the semilunar ganglion foramen to expose the roots of the trigeminal nerve. In these experiments where a portion of the ventral wall of the bulla was removed I usually obtained an infection within the middle ear, which developed into a good case of meningitis, killing the animal about the tenth day. This was prevented in the later experiments by placing a cigarette drain in the inner ear before sewing up the incision, and removing it a little each day after the third day. In the first experiments the trigeminal roots were severed by cutting them with a small curved scalpel, but since these roots were so intimately related to the circulus arteriosus (Willis) inwardly and to a venous sinus outwardly, it was extremely difficult to avoid injuring them. Better results were afterward obtained by using an electric cautery, the needle of which was bent at right angles. The best results were obtained in the last experiments by keeping out of the inner ear entirely and reaching the trigeminal roots with the curved electric needle through the semilunar ganglion foramen. This procedure required an exact knowledge of the course of the trigeminal roots, but no infection resulted in the middle ear, thereby eliminating the trouble of draining this cavity. A double closure was made of the incision, by first sewing together the fascia and lastly the skin. A dressing of iodine was finally applied to the wound.

The results of this experiment can be told by pricking the maxillary and mandible regions with a needle as soon as the animal has recovered from the effects of the anesthetic. If no response, the experiment has been successful. In this operation

it was impossible to destroy the sensory trigeminal root without destroying the motor root also, which of course produced a complete paralysis of the masticator muscles of the left side, so that it was necessary to nurse the animal for several days after the operation. The food selected was a saturated solution of cane-sugar in milk, which was administered through a pipette. In most cases a guinea-pig would regain the use of its jaws sufficiently to chew lettuce in a few days after the operation.

Microscopical technique. The animals were killed fourteen or fifteen days after the operation and the brains, including the trigeminal roots, were carefully removed and placed for a day or two in a 3 per cent potassium bichromate solution. Most of the cerebellum and the cerebral hemispheres were cut away from each brain, care being taken not to injure the remaining structures. The brain stem was then cut into three pieces, measured off so that the middle piece contained the trigeminal roots intact and extended cephalad to include most of the superior colliculus. The material was then changed to a fresh 3 per cent solution of potassium bichromate, where it remained for a period of three weeks. After rinsing with water, it was placed for three weeks in the following solution: 1 per cent osmic acid, 10 cc.; 3 per cent potassium bichromate, 30 cc. After this it was washed for twenty-four hours in running water; thoroughly dehydrated for a day in each grade of alcohol from 70 per cent up to 100 per cent; then to an alcohol-ether mixture for twenty-four hours, thin collodion three days, chloroform one hour, benzol two hours, benzol-soft paraffin twelve hours, medium (melting point about 50°C.) paraffin three hours, embedded in medium paraffin, cut into 20 μ sections, mounted serially on slides with the egg albumen method, cleared in xylol, damar, and cover-glass. According to my experience, there need be no haste with the dehydration process or with passing the material through ether, benzol, or xylol. After degenerated myelin is thoroughly blackened by osmic acid it is not affected by long exposure to these reagents, and one may as well have good sections as poor sections, as a result of a hurried infiltration process. For small brain sections the writer prefers the double collodion-paraffin

medium to either the collodion or paraffin alone. It has all the advantages of collodion plus the rapidity of the paraffin method.

For this study I had four complete series of guinea-pig brains, each having the left trigeminal roots severed behind the semilunar ganglion in the operation, but retaining proximally their attachment to the brain stem. The drawings were made from one series (experiment no. 58). They were outlined with the aid of a home-made drawing and projection apparatus (for description see the Anatomical Record for August, 1918). Every attempt was made to portray accurately the amount of nerve-fiber degeneration.

2. Results

If the left trigeminal roots from series no. 58 are examined caudad (centrally) to the lesion (fig. 11), it will be seen that the motor and sensory roots are sharply differentiated, not by connective tissue, but for the reason that there is no interchange of fibers. The sensory root (*S.R.V.*) is much the larger, more dorsal and lateral in position, and is completely filled with degenerated sensory fibers taking origin from cells in the semilunar ganglion. At this level the much smaller motor root (*M.R.V.*) is median and ventral to the sensory root. It contains a few degenerated fibers, but insufficient to be of any importance. I doubt if any of these are ascending mesencephalic root fibers taking origin from cells in the semilunar ganglion. Tracing these trigeminal roots caudad (centrally) to the exit of the motor fibers from the pons (fig. 9) shows no change in the sensory root (*S.R.V.*). The motor root (*M.R.V.*) has become separated from the sensory root and is embedded within the pons, but no more degenerated fibers have appeared among the motor fibers than were present more peripherally, demonstrating that no ascending mesencephalic root fibers have joined this root.

A more caudal section (fig. 8), at the cephalic end of the trigeminal motor nucleus, shows both trigeminal roots within the pons. The sensory root (*S.R.V.*) sends off many fibers and fine collaterals to the trigeminal sensory nucleus or substantia gelatinosa (*Sub.G.*). If an examination of the trigeminal motor

root is made between the trigeminal motor and sensory nuclei, it will be found to contain many degenerated ascending mesencephalic root fibers (*Mes.V.*). These fibers are central processes of semilunar ganglion cells, now leaving the trigeminal sensory root to enter the mesencephalic root, which at this level is mixed with the trigeminal motor fibers. In another series (ex. 51) where the dorsal half of trigeminal sensory root was cut, no degenerated fibers appeared in the mesencephalic root. This would indicate that the ascending sensory mesencephalic root fibers came from the mandibular, rather than from ophthalmic and maxillary portions of the trigeminal nerve. The importance of this observation will be considered under the discussion of the mesencephalic root at the end of the paper. A still more caudal section (fig. 5), passing through the caudal end of the trigeminal motor nucleus (*M.V.*), portrays many large and small degenerated fibers in the mesencephalic root (*Mes.V.*), which will be seen extending obliquely between the ventral border of the trigeminal sensory nucleus (*Sub.G.*) and the locus coeruleus (*Loc.C.*). It passes directly lateral to the trigeminal motor nucleus (*M.V.*) and sends to it many fibers and fine collaterals. A little below the locus coeruleus it also sends fibers and collaterals to a group of small cells situated median and dorsal to the trigeminal sensory nucleus. A portion of the mesencephalic root located above and lateral to the trigeminal motor nucleus in this section is shown more highly magnified in figure 6 so that every particle of degenerated myelin of any size could be accurately sketched. Observe the coarse degenerated fibers and fine collaterals in the mesencephalic root (*Mes.V.*), in the trigeminal motor nucleus (*M.V.*), and to the left of the mesencephalic root. A corresponding view of the right mesencephalic root (fig. 7) from the opposite or non-lesion side, shows no more degenerated fibers than would be found in any other area of this section not affected by this lesion or in a similar section from a normal brain.

It is clear from figures 5, 6, and 8 that the great bulk of the ascending mesencephalic root fibers, taking origin from cells in the semilunar ganglion, have ended in the trigeminal motor

nucleus or thereabouts. From figure 8 it is also apparent that a considerable number of these fibers have continued past the trigeminal motor nucleus and are traveling side by side with the descending mesencephalic root fibers in the region of the locus coeruleus (*Loc.C.*). This is more evident in figure 10, which includes the mesencephalic roots and locus coeruleus cells from the same section as figure 8, but more highly magnified to permit of the accurate drawing of every particle of degenerated myelin. The figure at the left is from the lesion side and a number of degenerated ascending sensory fibers will be seen in the mesencephalic root, while the right mesencephalic root (non-lesion side) does not contain any more degenerated fibers than an area a little median. In this figure the difference in number of degenerated fibers on the right and left sides represents the actual number of ascending sensory fibers in the mesencephalic root near the level of the exit of the trochlear nerve. The globular unipolar cells of the locus coeruleus (*Loc.C.*) are in marked contrast to the large multipolar cells of the trigeminal motor nucleus (fig. 6, *M.V.*).

There appears to be a gradual reduction in the number of degenerated fibers in tracing the left mesencephalic root cephalad through the inferior colliculus up to the level of the trochlear nucleus. In figures 12 and especially 13, which shows the mesencephalic root of figure 12 more highly magnified, it is perfectly evident that there are more coarse degenerated fibers in the left mesencephalic root (lesion side) than in the right. The difference in number will represent the number of ascending sensory fibers in the mesencephalic root at a level, slightly caudal, to the trochlear nucleus. It should be noted for this section that there are more fine particles of degenerated myelin scattered throughout the entire section than were present in more caudal sections. So far as could be determined, there were no more degenerated ascending mesencephalic root fibers on the operated side, cephalad of the trochlear nucleus, than on the opposite side, indicating that the ascending sensory fibers of the mesencephalic root did not extend cephalad of the level of this nucleus.

The other three series confirm the results recorded above. Also figure 19 shows more degenerated fibers in the left mesencephalic root than were present in the right. It is from a section at about the same level as figure 12, but from another experiment, where the left mesencephalic root was severed behind the inferior colliculus. This section is cephalad of the lesion and the degenerated fibers are ascending fibers, namely, the same sensory fibers as were cut in experiment no. 58, which had their cells of origin in the semilunar ganglion.

It is of interest to record in connection with figure 13 that the right trochlear nerve root(*IV*) contains more degenrated nerve fibers than the left. This section shows the trochlear roots after the fibers had crossed, so that the right root is really the left, which from its position (fig. 20, *IV*) would be severed with the trigeminal roots in experiment no. 58. This might signify that the trochlear nerve in the guinea-pig contains some scattered muscle sense ganglion cells, such as Nicholls described for the oculomotor nerve in sharks and in the frog, the central processes of which were severed in this experiment.

DESCENDING FIBERS IN THE MESENCEPHALIC ROOT

1. Distribution of the nervus trigeminus in the guinea-pig

It is obvious before much progress can be made in the solution of the distribution of the descending mesencephalic root fibers that an accurate knowledge should be obtained of the relationships of the various branches of the trigeminal nerve. With this in view, a careful dissection of this nerve was made in the guinea-pig. Also the physiological action of these nerves was tested by stimulating them with a weak induction current, the results of which will be recorded briefly before considering the experiments for determining the distribution of the descending mesencephalic root fibers.

As previously stated, the semilunar ganglion (fig. 3, *S.G.*) is situated within the skull directly above a large foramen in the alisphenoid (fig. 4, *S.F.*). The ganglion is more or less elliptical in shape, having its long axis parallel with the axis of the animal.

Its cephalic pole is continuous with the two large sensory components of the nervus trigeminus, the N. ophthalmicus and N. maxillaris (fig. 3, *Oph.* and *Max.*), both of which enter the orbit through the orbital fissure and the foramen rotundum, respectively. The N. mandibularis complex leaves the ventrolateral surface of the ganglion midway between the two poles, and from the caudal pole of the ganglion the trigeminal roots pass to the brain stem.

From figure 3 the mandibular nerve will be seen to have separated into three branches at its appearance from the semilunar ganglion. The most caudal of these is the nervus auriculotemporalis (*Aur.T.*), which is entirely sensory, pursues a general caudolateral course, at first caudal to the mandible, then penetrating the temporal muscle, innervates the skin immediately in front of the external auditory meatus. Stimulating this nerve with a weak induction current produced no contraction of the temporal muscle. Also serial sections from experiment no. 65, in which the trigeminal motor nucleus had been largely destroyed, demonstrated the absence of motor fibers in this nerve. The main division of the N. mandibularis (fig. 3, *Man.*) assumes a general ventral course median to the mandible. In the region of the angle of the mandible it divides into its three characteristic branches, the N. lingualis (*Lin.*) to the tongue, the N. alveolaris inferior (*Inf.A.*) to the mandibular canal, and the N. mylohyoideus (*Myloh.*) to the mylohyoid and digastric muscles. Stimulation of the mandibular nerve with a weak induction current produced a contraction of the digastric muscle. Also serial section from experiment no. 65 demonstrated degenerated motor fibers going to this nerve. The third division of the mandibular trunk, the N. masticatorius (fig. 3, *Mast.*), after leaving the semilunar ganglion lateral to the main mandibular nerve, passes in front of the mandible, to pursue a general lateral, ventral, and cephalic course. With the exception of the buccal branch, it is composed entirely of trigeminal motor fibers.[2] The N.

[2] In this description a nerve consisting purely of motor fibers means the entire absence of general cutaneous or somatic sensory fibers. Muscle sense is not considered.

buccinatorius (fig. 3, *Buc.*) happens to be the most cephalic branch of the masticator nerve. Curving around the inner surface of the temporal muscle, it enters the floor of the orbit a little median to the pterygoid nerve, where it branches ventrally to supply the mouth cavity. Serial sections from experiment no. 65 show no degenerated motor fibers in this nerve. Two Nn. temporales profundi appear directly behind the buccal nerve to innervate the temporal muscle. Only one of these nerves, the larger and most peripheral, is shown in figure 3 (*Tem.*). Immediately behind the deep temporal nerves there is a larger branch of the masticator, designated as the N. pterygoideus (fig. 3, *Pter.*). This nerve assumes a lateral and cephalic course, passing between the mandible and temporal muscle, enters the floor of the orbit a little lateral to the buccal nerve, and following along the dorsal surface of the pterygoid muscles, sends numerous branches to them. Before entering the orbit it gives off a branch to the masseter muscle. Some text-books on mammalian anatomy describe a similar nerve as the N. buccinatorius, but the nerve described above is purely a motor nerve, producing a contraction of the pterygoid muscles upon stimulation, and series no. 65 shows that it is full of degenerated motor nerve fibers. The N. masseter icus (fig. 3, *Mas.*) is the last branch of the masticator nerve. It is fully as large as the pterygoid nerve and, at first pursues a lateral cephalic course immediately behind the pterygoid nerve, and upon reaching the outer surface of the mandible, breaks up into several branches for the masseter muscle. Like the pterygoid and deep temporal nerves, it is purely a motor nerve as was shown by stimulation, and in tracing this nerve peripherally in series no. 65, branches containing degenerated motor fibers can be traced to masseter muscle fibers (fig. 30, *Mas.*).

To return to figure 3, where the purely sensory nerves are drawn in outline and the motor nerves are cross-barred, it will be seen that the ophthalmic and maxillary divisions of the trigeminus are sensory and the mandibular division is mixed. Of the three components of the mandibular, the masticator nerve is mainly motor (see previous footnote excluding muscle sense from this statement), only the small buccal branch being sensory; the

auriculotemporal division is entirely sensory, and the mandibular nerve proper is nearly all sensory, save a few motor fibers going to the mylohyoid and digastric muscles. The cell bodies from the fibers of the ophthalmic and maxillary divisions of the trigeminus, apparently, form the dorsal and median portion of the semilunar ganglion, and their central processes the dorsal portion of the sensory root, while the ventral and lateral cells in the semilunar ganglion have their central processes in the ventral part of the sensory root and their peripheral processes in the mandibular nerve. Close to its exit from the pons the trigeminal motor root is median to the sensory root, but gradually becomes ventral to it as it approaches the semilunar ganglion and passes through the ventral part of the semilunar ganglion. In the ganglion the motor root is separated from the cells and sensory fibers by a connective-tissue sheath (fig. 21, *C.T.*). The motor and sensory roots (fig. 28, *M.R.V.* and *S.R.V.*), while enclosed in a similar connective-tissue sheath, are not separated by connective tissue, and still there is no exchange of fibers between the two roots. The nervus trochlearis (fig. 20, *IV*) will be found in the connective-tissue sheath surrounding the two trigeminal roots.

2. Experiments

In these experiments it was required to sever the mesencephalic root at some point caudal to the inferior colliculus, without injuring in any way the trigeminal motor nucleus, roots, or nerves. The same general mode of procedure was applied in these experiments as was used in the first experiments of severing the trigeminal roots. The animals were allowed to live for the same length of time after the operation, and the brains were treated and sectioned serially after the same manner.

To be absolutely sure of my anatomical relationships, I removed the dorsal right half of the cranial wall from a formalin preserved specimen of about the same size as was to be used in the experiments. A median sagittal section was made through the brain with a sharp knife and the right half of the brain was completely removed, leaving the left half intact. This prepa-

ration proved to be very useful and was kept close at hand during all operations.

The operation for severing the mesencephalic root consisted in first making a longitudinal incision through the skin of the head and neck a little to the left of the median line and spreading out the skin laterally. Next the left temporal muscle was severed from its attachment to the sagittal crest, parietal and occipital bones and turned to the left side. A small opening was made through the left parietal with a pair of small bone forceps, extending in width from the median sagittal crest over to the left side about 5 mm., and of sufficient length to expose the left cerebral hemisphere. The line between the left cerebral hemisphere and the cerebellum is indicated by a depression, in which there is a transverse venous sinus of considerable size. If due care has been exercised not to cut the meninges, no hemorrhage will take place up to this point. Should one occur, it can be stopped with a little sterile bone wax. (Shortly before an operation I usually boil a little vaseline and beeswax, of equal proportions, in an aluminum crucible, which makes a very satisfactory wax upon cooling.) The instrument used for making the lesion consisted of a piece of steel shaped up into the form of a chisel, the two narrow sides of the blade were ground down so that the width of the blade, when placed over a section of the guinea-pig's brain stem in the region of the locus coeruleus, would equal the distance between the median line and the brachium conjunctivum. From the demonstration previously described, the necessary depth required to sever the mesencephalic root was figured out, measured off on the blade of the chisel, and indicated by a notch made with a file. Turning again to the demonstration, it was found that an incision made straight downward between the left cerebral hemisphere and the cerebellum, beginning at the median line, would sever the left mesencephalic root immediately behind the inferior colliculus. Since the transverse sinus is situated in this interval, some of the lesions were made through the cephalic end of the cerebellum directly behind the transverse sinus, and others were made through the caudal end of the left cerebral hemisphere, directing the path of the chisel straight downward or

slightly cephalad in the cerebellum incisions and slightly caudal in the cerebrum incisions. Care was taken not to let the edge of the chisel extend to the right of the median line, and the proper depth was indicated by the notch on the blade of the chisel. Good results were obtained from both incisions, but with little or no danger of injuring the trigeminal motor nucleus in the cerebellum incision if it happened to be a little too deep. If any hemorrhage resulted from the incision of the chisel, which in every case extended from the median line to the left side, it was stopped with a swab of hot sterile water. The left temporal muscle was put back in place and its superficial fascia was sewed to the superficial fascia of the right temporal muscle and the two flaps of skin were joined, producing a double closure over the aperture in the skull. A dressing of iodine was finally applied to the wound.

As in the previous experiments, the animals were allowed to live fifteen days before killing them and removing their brains. No infection occurred in any of these experiments, and it was found after killing the animal that the hole in the skull had completely ossified. Before removing a brain, all of the various branches of the trigeminal nerve, the semilunar ganglion, and the trigeminal roots from the left (lesion) side were carefully dissected away from the muscles and other structures, but their attachment to the brain was left intact. In several instances masseter muscle fibers were left attached to the masseter nerve, to be sure of its identification in sections.

After recovering from the shock of the operation, those animals in which the brachium conjunctivum was little injured, behaved exactly like normal animals. They would eat an equal amount of lettuce and tough carrots as rapidly as normal animals.

3. Results

Four series of section have been cut from this material. Each series included a part of the brain stem from a region a little behind the entrance of the trigeminal sensory root to sections passing through the cephalic end of the superior colliculus, with

both trigeminal roots attached on each side, together with the semilunar ganglion and all of the branches of the trigeminal nerve on the left (lesion) side. The series from experiment no. 64 demonstrated that the lesion, in addition to severing all of the mesencephalic root fibers taking origin from the mesencephalon, included at least one-half of the fibers originating from the locus coeruleus, with absolutely no damage to the trigeminal motor nucleus (figs. 14, 15, 17, and 18). In experiment no. 65 all of the mesencephalic root fibers from the midbrain and most of the locus coeruleus fibers were severed by this lesion, which also extended into the trigeminal motor nucleus, causing a degeneration of at least one-half of the motor root fibers. The lesion in experiment no. 67 was almost identical to experiment 64, except that it was a little more cephalad and severed fewer locus coeruleus fibers; while the lesion in experiment no. 68 was a little deeper than no. 64, severing more locus coeruleus fibers and coming very close to the trigeminal motor nucleus, which it may have injured, from the fact that a few degenerated fibers were found in the main mandibular trunk and none were present in series 64 and 67.

A careful study was made of all the series, which checked up identically in so far as the distribution of the mesencephalic root fibers was concerned. Most of the description and nearly all of the figures were taken from series no. 64 for the reason that we are absolutely certain in this experiment that all of the descending mesencephalic root fibers were severed without injury to the trigeminal motor nucleus or root. It will be seen from figure 18 that all of the descending mesencephalic root fibers (*Mes.V.*) taking origin from cells in the midbrain were severed by this lesion (*Les.*). From figures 14, 15, and 17 it is apparent that the lesion (*Les.*) passed through the locus coeruleus (*Loc.C.*), severing at least half of its fibers. From these figures, which also pass through different levels of the trigeminal motor nucleus (*M.V.*), it is perfectly evident that the lesion (*Les.*) does not come near enough to the nucleus to injure it. Series no. 65, in addition to being useful for tracing the distribution of the descending mesencephalic root fibers, enables one to determine for

a certainty, from sections, which of the trigeminal nerves contained motor fibers and which contained none. In this series the left trigeminal roots became curved outward somewhat during the process of fixation so that the roots and semilunar ganglion appear in almost perfect longitudinal section.

From series no. 64 it is apparent that the degenerated descending mesencephalic root fibers within the pons follow the descriptions of this root given by Johnston, and May and Horsley. After leaving the inferior colliculus a little lateral to the aquaeductus cerebri and median to the brachium conjunctivum, its fibers intermingle with the cells of the locus coeruleus and undoubtedly receive fibers from these cells. In fact, the cells of the locus coeruleus appear to be nothing more than a caudal continuation of the mesencephalic root cells of the midbrain. In the pons region the mesencephalic root (figs. 14, 15, and 17, *Mes.V.*) continues caudad through the locus coeruleus directly median to the brachium conjunctivum (*Br.C.*) and dorsal to the trigeminal motor nucleus (*M.V.*). At the caudal extremity of the trigeminal motor nucleus the degenerated descending mesencephalic root fibers (fig. 14, *Mes.V.*) bend lateral and ventral, to assume a cephalic course between the trigeminal sensory (substantia gelatinosa) and motor nuclei (figs. 15 and 17, *Mes.V.*). This cephalic arm of descending mesencephalic root fibers continues cephalad in the trigeminal motor root for some distance cephalad of the trigeminal motor nucleus. In figure 18, which is about twenty sections cephalad of the trigeminal motor nucleus, the trigeminal motor root with its numerous intermingled degenerated descending mesencephalic root fibers (*Mes. V.*) will be seen passing to the median side of the trigeminal sensory root (*S.R.V.*). Fifty-two sections cephalad, in figure 19, both motor and sensory roots (*M.R.V.* and *S.R.V.*) will be found outside the pons, and all of the degenerated descending mesencephalic root fibers are confined absolutely to the trigeminal motor root (*M.R.V.*). The truth of this statement can be easily confirmed by turning to figure 11, where the area of degenerated ascending fibers from the semilunar ganglion conforms exactly to the light area in figure 19 designated as the sensory root, and the

light area designated as the motor root (*M.R.V.*) in figure 11 is identical to the motor root in figure 19. Also the area of degenerated trigeminal motor fibers in figure 28 (*M.R.V.*), and better shown in more proximal sections of series 65, conforms exactly to the motor-root area of figure 19. A more detailed discussion of these roots is given below.

These results are in direct confirmation of May and Horsley's conclusions that the descending mesencephalic root fibers enter the motor root of the trigeminal nerve, and are opposed to Johnston's observations that they entered the trigeminal sensory root. Since Johnston worked only with normal brains stained after the Weigert method, he could have easily confused the descending mesencephalic root fibers with ascending sensory fibers entering the mesencephalic root, which are very numerous at this level (see previous description and fig. 8, *Mes.V.*).

The descending mesencephalic root fibers, like the ascending, send off collaterals to the trigeminal motor nucleus and to a group of cells situated a little median and dorsal to the trigeminal sensory root (figs. 15, 17, and especially 16). In the last-mentioned figure a portion of the mesencephalic root and the motor nucleus of the trigeminal nerve are shown more highly magnified to permit of an accurate drawing of every particle of degenerated myelin. A comparison of this figure with a similar drawing of the ascending fibers of the mesencephalic root (fig. 6, *Mes.V.*) shows at least twice as many descending mesencephalic root fibers in figure 16, but fewer fibers and collaterals (*Col.*) are given off to the trigeminal motor nucleus. An examination of the right mesencephalic root (non-lesion side) in this series demonstrates no more degenerated fibers than would be present in similar sections of a normal brain.

If an examination of the left trigeminal roots is made about half way between the exit of the motor root and the beginning of the semilunar ganglion (fig. 20), it will be seen that both roots are surrounded by the same connective-tissue sheath, which also contains the trochlear nerve (IV). The latter possesses many degenerated fibers and was undoubtedly severed, centrally, in making the lesion of the mesencephalic root. The motor root

(*M.R.V.*) has assumed a more ventral position than was shown in the more proximal section (fig. 19). The numerous degenerated descending mesencephalic root fibers in it, making up, to venture a guess, one-fifth or less of the total number of fibers, rendered the line of division sharply marked between the motor and sensory roots. Throughout the entire course of these roots there is absolutely no migration of descending mesencephalic root fibers from the motor root to the sensory root. Serial sections from experiments 67 and 68 show identically the same relationships between trigeminal motor and sensory roots and that the degenerated descending mesencephalic root fibers are confined solely to the motor root. In series no. 65, where the trigeminal motor nucleus was also partly destroyed in the lesion of the mesencephalic root, it is apparent in figure 28, which is a more or less longitudinal section through the trigeminal roots and the semilunar ganglion, that both trigeminal motor fibers and descending mesencephalic root fibers are confined solely to the motor root, and that there is absolutely no intermingling of motor fibers with the sensory fibers, even though there is no connective-tissue sheath separating them.

Figure 21 shows the position of the trigeminal motor root (*M.R.V.*) at about the center of the semilunar ganglion. Within the ganglion it is enclosed in a connective-tissue sheath (*C.T.*). There are fully as many degenerated descending mesencephalic root fibers in the motor root of this section as there were in figure 20. I followed the trigeminal motor root section by section, not only in this series, but also in series 67 and 68, and found no descending mesencephalic root fibers leaving the motor root to go to the ganglion. Also figure 28, which happens to be a longitudinal section through the trigeminal motor root and the semilunar ganglion, demonstrates that the motor root passes straight through the ganglion without giving off any descending mesencephalic root fibers or any trigeminal motor fibers to the ganglion. It is perfectly clear that the descending mesencephalic root fibers do not end in the semilunar ganglion as May and Horsley supposed, but continue straight through it in the motor root to enter the nervus masticatorius.

A section directly peripheral to the semilunar ganglion (fig. 22) shows that the motor root of the trigeminal nerve had migrated laterally through the peripheral processes of the semilunar ganglion to become the motor component of the nervus masticatorius (*Mast.*), dividing the sensory nerve processes into a cephalic and a caudal portion. Also that the cephalic portion of these fibers has been subdivided by connective tissue into the nervus ophthalmicus (*Oph.*) and a much larger portion, the N. maxillaris (*Max.*). Likewise, the caudal portion of these sensory fibers has been subdivided into the N. auriculotemporalis (*Aur. T.*) and the large N. mandibularis proper (*Man.*), which is situated directly medial to the motor division of the trigeminal nerve, namely, the N. masticatorius (*Mast.*). In passing between these two bundles of sensory nerves, the motor root gave off a few motor fibers to the N. mandibularis (fig. 29, *Man.*) and received a few sensory fibers in return to form the buccal branch (fig. 22, *Buc.*) of the N. masticatorius. From figure 22 and the above description, the writer would maintain that, in the guinea-pig, the auriculotemporal and masticator nerves are no more branches of the mandibular nerve than the ophthalmic nerve is a branch of the maxillary. All five nerves appear to arise separately from the general complex of fibers at the distal end of the semilunar ganglion. If muscle sense is eliminated, the ophthalmic, maxillary, and auriculotemporal nerves are sensory, the mandibular is sensory save for the mylohyoid, and the masticator, motor, except for the buccal branch.

It is perfectly clear from figure 22 that all of the degenerated descending mesencephalic root fibers went to and were contained in the masseter (*Mas.*), pterygoid (*Pter.*), and deep temporal (*Tem.*) branches of the N. masticatorius, which were listed in the previous paragraph as motor nerves. Absolutely no more degenerated fibers are to be found in the N. auriculotemporalis (*Aur.T.*), N. mandibularis proper (*Man.*), N. maxillaris (*Max*). and N. ophthalmicus (*Oph.*) than would be found in a corresponding section of a perfectly normal trigeminal nerve. A similar section from series 67 would also demonstrate that all of the degenerated descending mesencephalic root fibers are con-

fined to the motor branches of the N. masticatorius. Figure 29 shows that all of the degenerated fibers, representing both descending mesencephalic root fibers and trigeminal motor fibers, are present in the N. mandibularis proper (a few motor fibers for the mylohyoid nerve), masseter, pterygoid and deep temporal branches of the N. masticatorius. Likewise, a similar section from series 68 would show a few degenerated fibers, probably motor, in the N. mandibularis proper. The great bulk of degenerated fibers are in the so-called purely motor branches of the N. masticatorius.

A more peripheral section from this series (fig. 23) exhibits the five main branches of the trigeminal nerve more widely separated, and a portion of this section is sufficiently magnified in figure 24 so that every particle of degenerated myelin of any size could be accurately represented. This figure includes the nervus auriculotemporalis (*Aur.T.*), the main portion of the N. mandibularis (*Man.*), the N. masticatorius (*Mast.*), and representative bundles (a) and (b) from the N. ophthalmicus and N. maxillaris. As in figure 22, the degenerated descending mesencephalic root fibers are confined solely to the masseter (*Mas.*), pterygoid (*Pter.*), and deep temporal (*Tem.*) branches of the N. masticatorius. Absolutely no more degenerated fibers are to be found in the N. auriculotemporalis, the main trunk of the N. mandibularis, the buccal (sensory) branch of the N. masticatorius, and bundles (a) and (b) from the N. ophthalmicus and N. maxillaris than would appear in a section of a perfectly normal trigeminal nerve. Since the N. masticatorius has given off but one small branch to the temporal muscle, there are apparently fully as many normal and degenerated nerve fibers in the motor branches of the N. masticatorius as there were in more central sections. Also about the same ratio of normal to degenerated fibers persists. Of the various motor nerves in the N. masticatorius shown in figure 24, the deep temporal (*Tem.*) is much the smallest and the masseter (*Mas.*) is a little larger than the pterygoid (*Pter.*). All possess relatively about the same number of degenerated descending mesencephalic root fibers. If there is any difference, the N. pterygoideus has the greatest number in proportion to its size.

Figures 25 and 26 are more peripheral sections of the same series, no. 64. They exhibit the same general arrangement of the trigeminal nerve, but the various nerves are more scattered and the nervus ophthalmicus does not appear in these sections. In figure 25 the most peripheral of the two deep temporal nerves (*Tem.*) will be seen leaving the N. masticatorius for the temporal muscle. Also the masseter nerve (*Mas.*) is about to give off branches to the masseter muscle. As in the previous sections, degenerated descending mesencephalic root fibers appear only in the masseter, pterygoid, and deep temporal branches of the N. masticatorius (*Mast.*). In the more peripheral section (fig. 26), the buccal branch (*Buc.*) of the masticator nerve has left the main trunk; only the masseter and pterygoid branches remain. Figure 27 shows the N. masticatorius from the same section as figure 26 sufficiently magnified to permit of the accurate drawing of every particle of degenerated myelin. Thus far, the pterygoid nerve (*Pter.*) has not branched and contains about the same number of degenerated descending mesencephalic root fibers as it did in a more proximal section (fig. 24). On the other hand, the masseter nerve (*Mas.*) has branched several times and is appreciably smaller, but still maintains about the same ratio of degenerated to normal fibers in the main stem and its branches. In this section (fig. 27) the masseter and pterygoid nerves are about the same size, but the pterygoid has a few more degenerated descending mesencephalic root fibers.

Similar sections from the left trigeminal nerve from series no. 67 could have been selected to demonstrate that the distribution of the descending mesencephalic root fibers is confined solely to the so-called purely motor branches of the N. masticatorius. In this experiment a few of the masseter muscle fibers were left attached to the masseter nerve upon removing it from the animal, and in section it was possible to trace degenerated descending mesencephalic root fibers to a branch of the masseter nerve going to these muscle fibers. Also in the lower part of figure 30, which is from a peripheral section from series no. 65, where the descending mesencephalic and many trigeminal motor root fibers were severed, a branch from the masseter nerve (*Mas.*),

containing both degenerated descending mesencephalic and motor root fibers, will be seen going to a bundle of masseter muscle fibers (*M.F.*). The excess number of degenerated fibers in the masseter nerves in figure 30 over figure 27 represent trigeminal motor fibers destroyed by the lesion in experiment no. 65. Sections of the trigeminal nerves in series no. 68 are almost identical to series 64 and 67, except that a few degenerated fibers, probably trigeminal motor fibers, appear in the main mandibular trunk.

It can be stated for experiments 64, 65, 67, and 68 that no descending mesencephalic root fibers were found outside the mandibular division of the trigeminal nerve. Also from experiments 64 and 67, where the mesencephalic root was severed with absolutely no damage to the trigeminal motor nucleus or to the roots of the trigeminal nerve, it is perfectly clear that the descending mesencephalic root fibers are confined solely to the so-called purely motor branches of the nervus masticatorius, namely, the masseter, pterygoid, and deep temporal nerves.

Returning again to the results of the experiments in which the trigeminal roots were severed centrally to the semilunar ganglion, where it was found that a considerable part of the mesencephalic root was composed of sensory fibers taking origin from the semilunar ganglion cells and also to experiment 51, where no degenerated ascending sensory fibers were found in the mensencephalic root after a lesion of the dorsal part of the trigeminal sensory root, which fibers were undoubtedly from the ophthalmic and maxillary branches of the trigeminus (purely sensory nerves), it is fair to assume that the so-called ascending sensory mesencephalic root fibers, like the descending mesencephalic root fibers, were distributed to the masticator muscle nerves. Since no decending mesencephalic root fibers went to the mylo-hyoid and digastric nerves, it is quite probable that the ascending sensory fibers of the mesencephalic root supply the mylo-hyoid and digastric muscles.

So far as I am aware muscle sense from the masseter muscle could not reach the trigeminal nerve by any other way than the N. masticatorius, and it has been demonstrated that no fibers

from the masseter branch of this nerve go to the semilunar ganglion or to the sensory root. It is no more surprising to find muscle sense fibers in the purely motor components of the trigeminus than to find them in the oculo-motor nerve.

NEURONS OF THE MESENCEPHALIC ROOT

In order that the cells of origin of the descending mesencephalic root fibers within the inferior colliculus and locus coeruleus might be studied and compared with the sensory cells of the semilunar ganglion and the motor cells of the trigeminal motor nucleus, two guinea-pig brains with their left trigeminal roots and ganglions intact were prepared after a slight modification of the Cajal-Ranson silver method and were sectioned serially. The technique employed was as follows: Material was placed for two days in a mixture of equal parts of the following solutions: a) 100 cc. absolute alcohol plus 1.5 cc. strong ammonia; b) 100 cc. of 10 per cent formalin plus 3 cc. strong ammonia. Removed to running water for twenty-four hours. Placed in pyridin for twenty-four hours, dilute pyridin and running water for twenty-four hours. Stained in 2 per cent silver nitrate for seven days in an oven at 38°C. Reduced with a 2 per cent hydrochinon solution plus 5 cc. of formalin to every 100 cc. of hydrochinon for twenty-four hours in an oven. Embedded after the collodion-paraffin method and the sections were cut 20μ thick.

Figure 31a shows one of the ordinary semilunar ganglion cells to be identical with one of the large spinal ganglion cells with a coiled process. In figures 31b and 32 we have a cell from the locus coeruleus and two mesencephalic root cells from the inferior colliculus, all drawn to the same scale of magnification as figure 31a. It is perfectly evident that the mesencephalic root and locus coeruleus cells are globular unipolar cells of the same size and type as the sensory cells of the semilunar ganglion, the only difference being that their single process is not coiled about itself If, on the other hand, a comparison is made with the much larger multipolar cells of the trigeminal motor nucleus (figs. 33e and 35), little in common will be found between these

two types of cells, even though the locus coeruleus cells may be located directly above the trigeminal motor nucleus. The writer agrees with Johnston that the position of the globular unipolar mesencephalic root cells in the alar (sensory) plate of the midbrain is sufficient reason for classifying the descending mesencephalic root fibers as sensory rather than motor.

As previously stated, the locus coeruleus cells appear to be nothing more than a caudal extension of the mesencephalic root cells downward into the motor area of the pons. A study of their embryology would likely show that the locus coeruleus cells also came from the alar plate. In these sections numerous fibers and collaterals were seen to go from the region of the mesencephalic root toward the trigeminal motor nucleus; such a collateral is shown in fig. 34, *Col.* These collaterals appear to be especially numerous in the region where the mesencephalic root fibers bend ventrally around the caudal end of the trigeminal motor nucleus, but it is necessary to search many fibers before one is found that has a collateral. Most of such pictures turn out to be merely the crossing of these fibers over or under the mesencephalic root fibers.

GENERAL SUMMARY, DISCUSSION AND CONCLUSIONS

The results of the previous experiments confirm May and Horsley's conclusions that the mesencephalic trigeminal root contains both ascending and descending fibers. The former take origin from sensory cells in the semilunar ganglion and the latter from globular, unipolar cells in the alar (sensory) plate of the mesencephalon, and from a caudal continuation of these cells, known as the locus coeruleus, which extend downward into the motor area of the pons.

Upon emerging from the inferior colliculus the mesencephalic root is composed mainly of descending fibers. They continue caudad through the locus coeruleus above the trigeminal motor nucleus, bend laterally and ventrally around the caudal end of this nucleus, to pursue a cephalic course between the trigeminal motor and sensory (substantia gelatinosa) nuclei, and here inter-

mingle with the trigeminal motor root fibers, which follow along the ventral surface of the trigeminal sensory root, through the ventral border of the semilunar ganglion, to eventually form the motor components of the nervus masticatorius, for the masseter, pterygoid, and temporal muscles. None of the descending mesencephalic root fibers followed the few motor fibers into the mylohyoid branch of the nervus mandibularis, nor entered the purely sensory divisions of the trigeminal nerve. It will be seen that these results confirm May and Horsley in that the descending mesencephalic root fibers enter the trigeminal motor root, but disagree with them, that they end in the semilunar ganglion. It is apparent that their studies in chromatolysis, which were at variance and held in subjection to their results from a study of Marchi preparations, were in accord with my results from Marchi preparations.

The ascending mesencephalic root fibers taking origin from sensory cells in the semilunar ganglion follow along in the ventral half of the trigeminal sensory root. Upon entering the pons they join with the descending mesencephalic root fibers to form the mesencephalic root or tract. In passing between the trigeminal motor and sensory nuclei they appear to make up about one-third of the mesencephalic root. Since a large number of these fibers and collaterals go to the trigeminal motor nucleus, the ratio of ascending to descending fibers in the mesencephalic root cephalad of the trigeminal motor nucleus is reduced something like 1 to 7 or 8. The number of ascending fibers continues to decrease gradually as the mesencephalic root passes through the inferior colliculus, until no ascending fibers were found in the mesencephalic root cephalad of the trochlear nucleus.

Without a knowledge of the character and position of the cell bodies of the descending mesencephalic root fibers, it might be assumed, since these fibers entered the motor root and traveled in the so-called purely motor divisions of the trigeminal nerve, that they were motor fibers. The writer, after making a careful study of these globular unipolar mesencephalic root cells, noting their position in the alar (sensory) plate, and comparing them with both the sensory cells of the semilunar gan-

glion and the motor cells of the trigeminal motor nucleus, agrees with Johnston and Willems that they are sensory rather than motor and favors Johnston's hypothesis that they represent neural crest cells, which were not extruded or possibly were extruded and later pulled back, when the medullary folds of the midbrain rolled up to form a tube. So far as known, there would be no need in the trigeminal nerve for a special motor nucleus, like the salivatory nuclei of the facial and glossopharyngeal nerves; the trigeminal motor nucleus should be fully capable of regulating the contractions of the masticator muscles.

Granting that the descending mesencephalic root fibers are sensory, it follows from their distribution that they are not carriers of cutaneous sensations, else they would be distributed to the maxillary, ophthalmic, and sensory branches of the mandibular nerve; hence they must be muscle sense fibers. In fact, it is difficult to conceive how muscle sense, say from the masseter muscle, which surely must possess muscle sense endings, could reach the trigeminal nerve other than through the masseter branch of the nervus masticatorius, which fibers we have shown do not enter the semilunar ganglion nor the trigeminal sensory root.

There can be no question but that the ascending mesencephalic root fibers are sensory. Since they were not found in the dorsal part of the sensory root, they probably did not come from the ophthalmic and maxillary (general cutaneous) divisions of the trigeminal nerve. More than likely, the ascending mesencephalic root fibers are also carriers of muscle sense. Inasmuch as no descending mesencephalic root fibers were present in the nervus mandibularis proper in the guinea-pig and Willems found no chromatolysis of the mesencephalic root nucleus cells after severing the mylohyoid and digastric nerves, it is quite possible that these ascending fibers came from the mylohyoid and digastric muscles, and entered the main mandibular nerve through the nervus mylohyoideus. It should be noted that some of the degenerated ascending fibers seen in the mesencephalic root as it passes between the trigeminal motor and sensory nuclei may be cutaneous fibers and collaterals from the sensory nucleus going to the motor nucleus.

It was stated that many fibers and collaterals from both the ascending and descending mesencephalic root fibers went to the trigeminal motor nucleus and to a group of small cells situated medial and dorsal to the trigeminal sensory nucleus. The former evidently form reflex arcs with the motor cells and the latter may be a muscle sense relay station (?) to the cerebral cortex, like the sensory nucleus (substantia gelatinosa) for the cutaneous sense fibers.

Little was accomplished in the way of observations on animals for the reason that there were many obstacles encountered in making satisfactory lesions that would destroy all muscle sense fibers going to the masticator muscles. To be absolutely certain of accomplishing this, it would be necessary to destroy both semilunar ganglions and the motor roots passing through them or the equivalent, which would of course result in a complete paralysis of the masticator muscles. In those experiments where the left mesencephalic root was severed in the pons, with little damage to the brachium conjunctivum, no difference could be detected in the action of the left masticator muscles in these animals and those of normal animals. Something interesting might result in severing both mesencephalic roots in the pons of an animal and testing its ability to compete with a normal animal in eating blindfolded a mixture of lettuce and tough roots. Also muscle spindle preparations from the masseter muscles of an animal having its mesencephalic roots severed in the pons would likely show some degeneration.

The results of the previous experimental work on the mesencephalic root, which have been obtained mainly from counting the chromatolytic cells in the mesencephalic root nucleus after severing certain branches of the trigeminal nerve, may be summarized as follows: For the rabbit Willems and Kosaka found no chromatolytic cells in the mesencephalic root nucleus, except after severing the N. mandibularis or its motor branches. In the dog Kosaka found a few degenerated cells in the mesencephalic root nucleus after destroying the N. maxillaris and certain sensory branches of the N. mandibularis. In the monkey May and Horsley found absolutely no chromatolysis in any of

the mesencephalic root cells after a lesion of the N. ophthalmicus and N. maxillaris; while Kosaka describes a few disintegrated cells in the mesencephalic root nucleus following a lesion of the N. maxillaris. Of these three investigators Kosaka is the only one to claim any general cutaneous fibers arising from the mesencephalic root nucleus and his results in the monkey are diametrically opposed to those of May and Horsley.

It is possible that there may be some variation in different vertebrates concerning the distribution of the fibers from the mesencephalic root nucleus and that some animals may have an appreciable general sensory component, but the weight of evidence thus far favors the conclusion that the descending fibers of the mesencephalic root are concerned only with muscle sense. This is certainly true for the guinea-pig, where after a lesion of the mesencephalic root in the pons, the degenerated fibers were traced in a series of Marchi sections solely to the masticator muscle branches of the N. masticatorius. It might be mentioned in this connection that degeneration shown by the Marchi method is more convincing than a few disintegrated cells in central nucleus as resulting from a peripheral lesion of its fibers. For the chromatolysis method to be conclusive a number of cells should be shown to have undergone degeneration.

From the data at hand the writer would regard the mesencephalic root as the muscle sense portion of the trigeminal nerve, having its ganglion cells located both in the semilunar ganglion, and in the midbrain and locus coeruleus. The peripheral processes of the latter constitute the so-called descending fibers, which follow the trigeminal motor root fibers into the masseter, pterygoid, and temporal branches of the nervus masticatorius. The peripheral processes of these semilunar ganglion cells may go through the mylohyoid branch of the nervus mandibularis to the mylohyoid and digastric muscles; while their central processes enter the pons through the trigeminal sensory root and constitute the so-called ascending fibers of the mesencephalic root. Fibers and collaterals from this system are distributed to the trigeminal motor nucleus and to a group of small cells situated medial and dorsal to the trigeminal sensory nucleus.

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EXPLANATION OF THE PLATES

ABBREVIATIONS

a., strand of the N. ophthalmicus
Al., os alisphenoidale (ala magna)
Aq., aquaeductus cerebri (Sylvii)
Aur. T., nervus auriculotemporalis
b., strand of the N. maxillaris
Br. C., brachium conjunctivum
Buc., nervus buccinatorius
Ch., chonae
Col., collaterals
C. T., chorda tympani
Gl., glioma
Inf. A., nervus alveolaris inferior
J. F., jugular foramen
Les., lesion
Lin., nervus lingualis
L. Lem., lemniscus lateralis
Loc. C., locus cœruleus
Man., nervus mandibularis
Mas., nervus massetericus
Mast., nervus masticatorius
Max., nervus maxillaris
Mes. C., mesencephalic root cell
Mes. V., radix mesencephalica trigemini
M. F., muscle fiber
M. L. B., fasciculus longitudinalis medialis
M. Lem., lemniscus medialis
M. R. V., trigeminus motor root
M. V., trigeminus motor nucleus
Myloh., nervus mylohyoideus
O. F., orbital fissure
O. N., ovale notch (partial foramen)
Oph., nervus ophthalmicus
Pter., nervus pterygoideus
S. F., Semilunar ganglion foramen
S. G., semilunar ganglion
S. M. F., stylomastoid foramen
Sph. P., nervus sphenopalatinus
S. R. V., trigeminus sensory root
Sub. G., trigeminus sensory nucleus or substantia gelatinosa
T., tympanic bulla
Tem., nervus temporalis profundus
IV., nervus trochlearis
VII., facialis root
XII., nervus hypoglossus

PLATE 1

EXPLANATION OF FIGURES

1 Transverse section through the brain stem of a small child at the level of the caudal end of the inferior colliculus (corpora quadrigemina), treated after a modified Marchi method for demonstrating degenerated medullary sheaths. Observe the size of the glioma, which was still larger more caudally, having involved the trigeminal roots on both sides in their course through the brachium pontis. The marked degeneration of the mesencephalic and troclear roots shown in this figure and in figure 2 cannot be attributed solely to a degeneration process, progressing centrally, as might be surmised from the fact that the region of the cells of origin of these two roots had not been invaded by the tumor. × 1.2.

2 Portion of the left mesencephalic root from the same section as figure 1. but more highly magnified to show the practically complete degeneration of the fibers of the mesencephalic root. × 4.8.

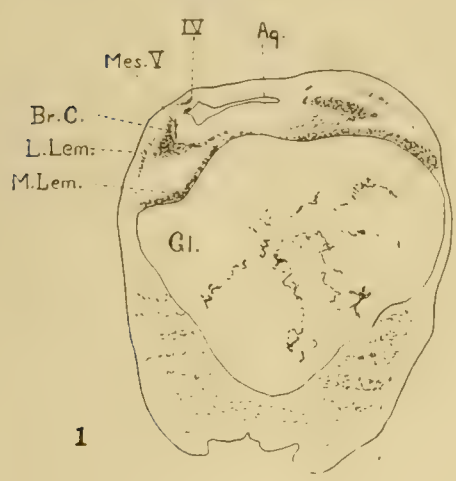
3 Dissection of the left trigeminal nerve of a guinea-pig. The sensory components are drawn in outline and the motor are cross-barred. There is some doubt as to the identification of the chorda tympani, but a nerve so designated was found leaving the lingual nerve close to its union with the inferior alveolar and to pass toward the tympanic bulla. × 1.6.

4 Photograph of the ventral surface of a guinea-pig's skull to show the large foramen in the alisphenoid, designated as the semilunar ganglion foramen, through which the trigeminal roots can be destroyed without the necessity of drilling through the skull. × 4/5.

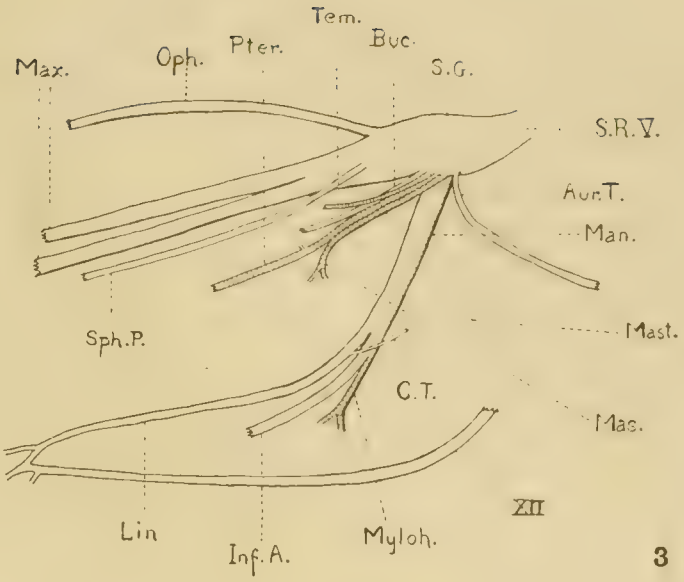
5 to 13 are from a transverse series of 20μ sections through a brain stem of a guinea-pig (experiment no. 58), in which the left trigeminal roots had been completely severed by an electric cautery immediately behind the semilunar ganglion, and the material to be sectioned was treated after a modified Marchi method for demonstrating medullary sheath degeneration.

5 Left half of a transverse section through the brain stem of a guinea pig (ex. 58) at the level of the caudal end of the trigeminal motor nucleus. Observe the complete degeneration of the trigeminal sensory root and the fine collaterals in its sensory nucleus (substantia gelatinosa). Between the trigeminal sensory and motor nuclei will be seen numerous degenerated ascending mesencephalic root fibers, which took origin from cells in the semilunar ganglion and from which many fibers and fine collaterals are sent to the trigeminal motor nucleus and to a group of smaller cells situated directly above and median to the sensory nucleus. × 8.

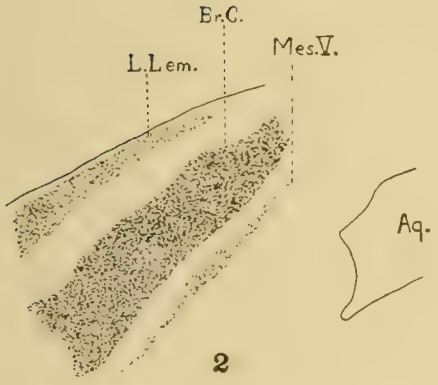
6 Portion of the left mesencephalic root from the same section as figure 5 more highly magnified so that every particle of degenerated myelin of any size could be accurately sketched. × 48.


IV
Aq.
Mes.V
Br.C.
L.Lem.
M.Lem.
Gl.

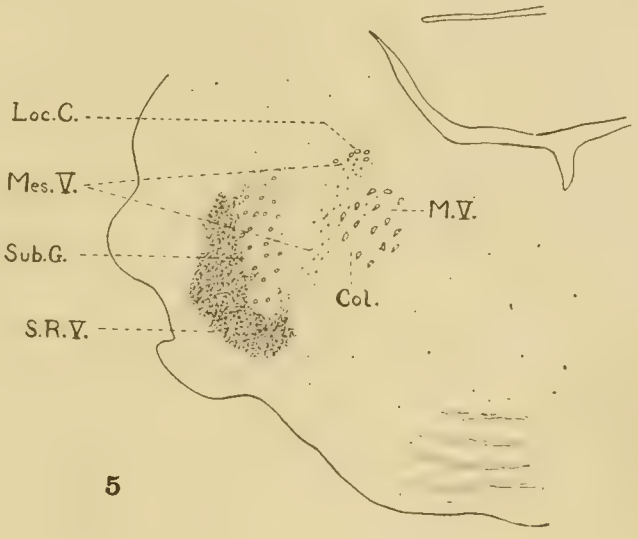
1


Tem.
Max.
Oph.
Pter.
Buc.
S.G.
S.R.V.
Aur.T.
Man.
Mast.
Sph.P.
C.T.
Mas.
XII
Lin
Inf.A.
Myloh.

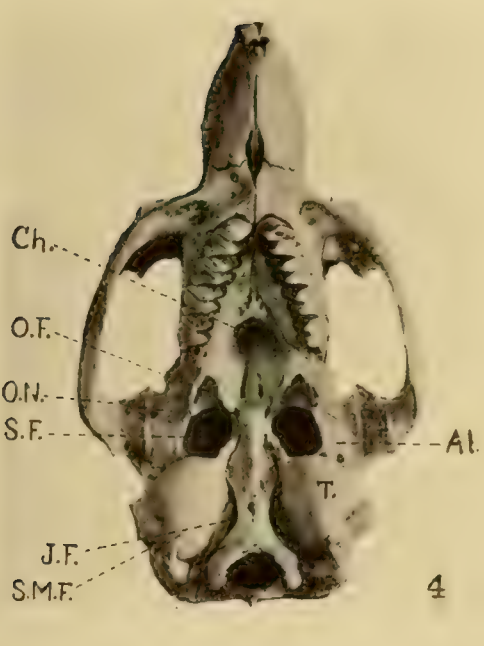
3


Br.C.
L.Lem.
Mes.V.
Aq.

2


Loc.C.
Mes.V.
M.V.
Sub.G.
Col.
S.R.V.

5


Ch.
O.F.
O.N.
S.F.
Al.
T.
J.F.
S.M.F.

4


Col.
Col.
Mes.V.
M.V.

6

PLATE 2

EXPLANATION OF FIGURES

7 Portion of the right mesencephalic root from the same section as figure 5, but from the opposite or non-lesion side. A comparison with figure 6 shows a great reduction in the number of degenerated fibers. No more are present than would appear in any other field of this section not affected by this lesion or would occur in a similar section of a normal brain. × 48.

8 Left half of a transverse section from the same series as figure 5 (ex. 58), but at the level of the cephalic end of the trigeminal motor nucleus. Observe the complete degeneration of the trigeminal sensory root and the fine collaterals in the trigeminal sensory nucleus (substantia gelatinosa). Degenerated ascending mesencephalic root fibers appear in transverse section in the locus cœruleus and intermingled with the trigeminal motor root fibers midway between the trigeminal sensory and motor nuclei. A few degenerated mesencephalic root fibers and collaterals will be seen in the trigeminal motor nucleus. × 8.

9 Similar transverse section to figure 8, but nineteen sections cephalad. This section is at a higher level than the trigeminal motor nucleus and shows a number of degenerated ascending mesencephalic root fibers in the locus cœruleus directly median to the brachium conjunctivum. The trigeminal motor root is about to join the sensory root from the median side. It contains a few degenerated fibers, but too few to be of any significance. × 8.

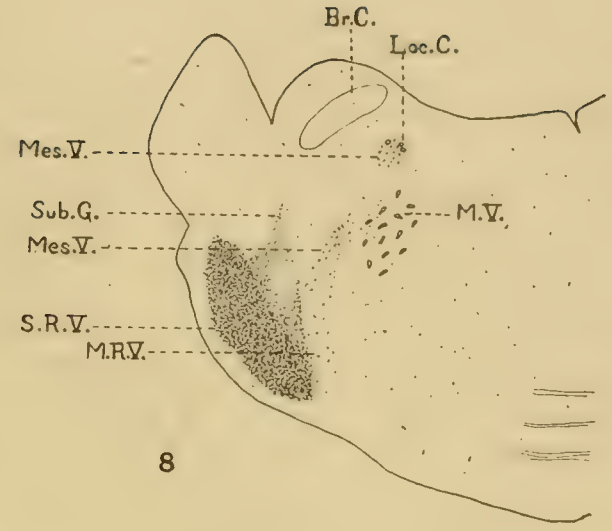
10. Mesencephalic roots and locus cœruleus cells from the same section as figure 9 sufficiently magnified so that every particle of degenerated myelin could be accurately sketched. Note that no more degeneration is shown in the right mesencephalic root (non-lesion side) than occurs outside this tract, while considerable degeneration is present on the left (lesion) side and many of the degenerated fibers are of large size. × 48.

11 Left half of a transverse section through the same guinea-pig's brain as figures 1 to 10 (ex. 58), but near the level of the caudal end of the inferior colliculus (corpora quadrigemina) at the exit of the trochlear nerves. Observe the mesencephalic root in transverse section directly median to the brachium conjunctivum. There are fewer degenerated fibers in the mesencephalic root than in the section from which figures 9 and 10 were drawn, which is twenty-eight sections further caudad. As was noted for figure 9, there are a few degenerated fibers in the trigeminal motor root, which at this level is ventral as well as median to the sensory root. × 8.

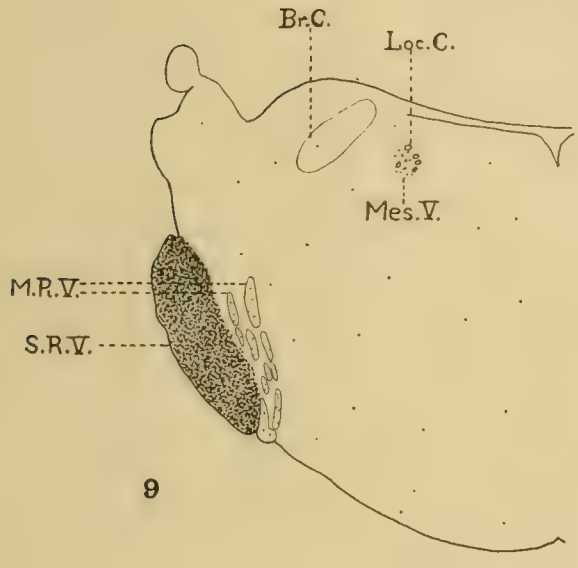
12 Similar view of a transverse section from the same series as figure 11, but through the inferior colliculus a little caudal to the trochlear nuclei. Note a few coarse degenerated fibers and a number of fine particles of myelin in the mesencephalic root. × 8.


M.V.
Mes.V.

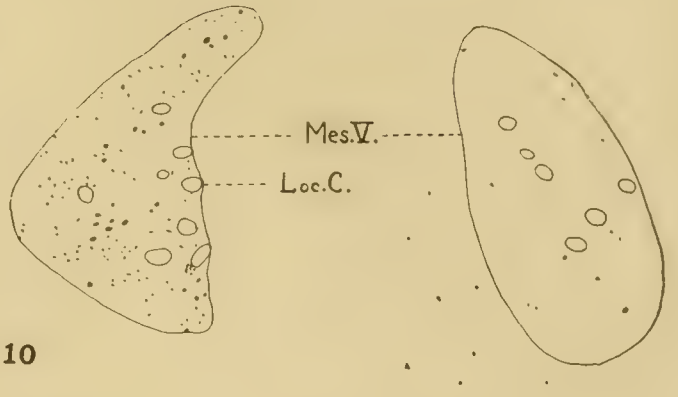
7


Br.C.
Loc.C.
Mes.V.
Sub.G.
M.V.
Mes.V.
S.R.V.
M.R.V.

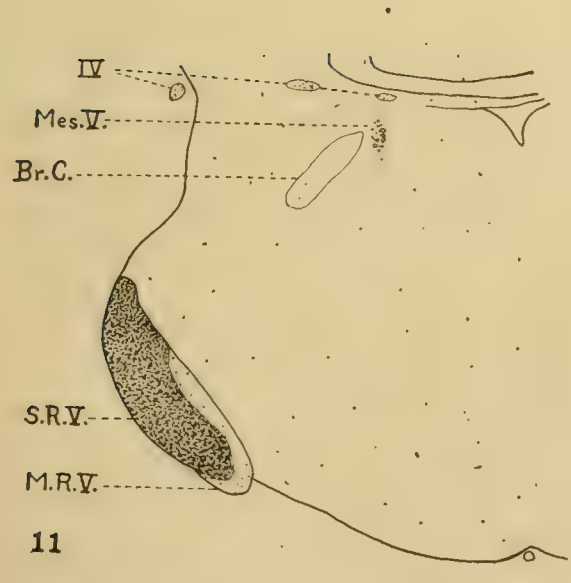
8


Br.C.
Loc.C.
Mes.V.
M.R.V.
S.R.V.

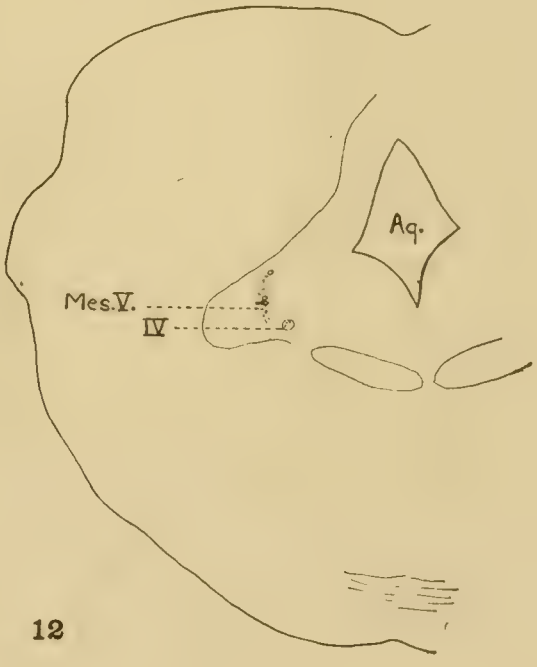
9


Mes.V.
Loc.C.

10


IV
Mes.V.
Br.C.
S.R.V.
M.R.V.

11


Aq.
Mes.V.
IV

12

PLATE 3

EXPLANATION OF FIGURES

13 Mesencephalic and trochlear roots from the same section as figure 12, sufficiently magnified to allow accurate drawing of every particle of degenerated myelin of any size. In both mesencephalic roots and elsewhere in this section there are many more fine particles of myelin than appear in more caudal sections. Observe in this figure that there are more coarse degenerated myelin sheaths in the right trochlear root than in the left. Since the fibers of these roots have crossed, the right root represents fibers for the left trochlear nerve. Its close proximity to the trigeminal roots probably resulted in its destruction. × 48.

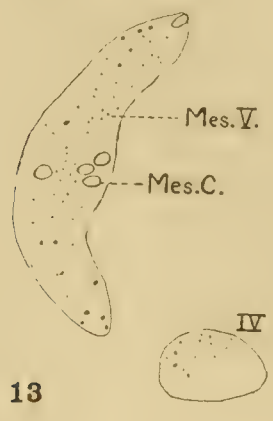
14 to 27 are from a transverse series of 20μ sections through a guinea-pig's brain stem, including the trigeminal roots, semilunar ganglion, and the trigeminal nerves (ex. no. 64). The lesion in this experiment passed completely through the left mesencephalic root behind the inferior colliculus and destroyed about one-half of the locus cœruleus, without disturbing the trigeminal motor nucleus nor any of its fibers. The sections were prepared after a modified Marchi method for demonstrating medullary-sheath degenerations.

14 Left half of a transverse section from the above-mentioned guinea-pig series (ex. 64) at the level of the extreme caudal end of the trigeminal motor nucleus. The lesion (*Les.*), which extends ventrocephalad, does not pass deep enough in this section to reach the mesencephalic root and the locus cœruleus, and is some distance from the trigeminal motor nucleus. Observe the almost complete degeneration of the fibers of the mesencephalic root, which curve around the caudal end of the trigeminal motor nucleus. A few fine mesencephalic root collaterals will be seen in the trigeminal motor nucleus. The globular unipolar cells of the locus cœruleus are some distance from the much larger multipolar cells of the trigeminal motor nucleus. × 8.

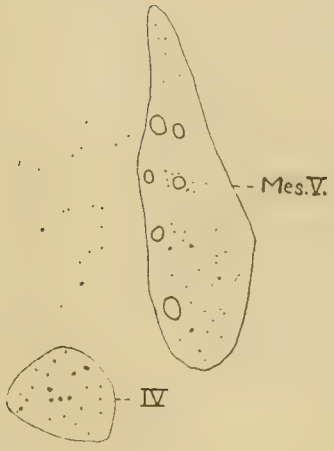
15 Similar view of a section ten sections cephalad of figure 14, passing through the caudal end of the trigeminal motor nucleus. The lesion (*Les.*) is a little deeper than in the previous section, severing some of the mesencephalic root fibers, but quite remote from the trigeminal motor nucleus. In this section the mesencephalic root will be seen in transverse section in the locus cœruleus a little median to the brachium conjunctivum, and also as longitudinal fibers, bending ventrally, between the trigeminal sensory and motor nuclei. Collaterals pass to the trigeminal motor nucleus and to some smaller cells situated median and dorsal to the trigeminal sensory nucleus. × 8.

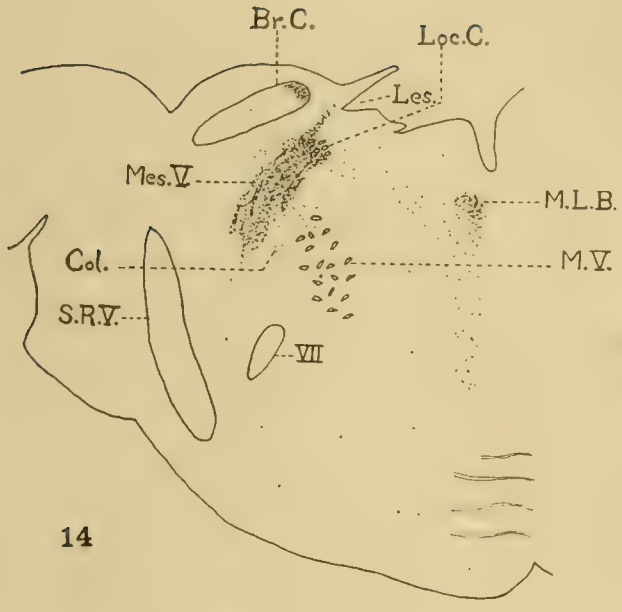
16 Portion of the left mesencephalic root between the trigeminal sensory (substantia gelatinosa) and motor nuclei from the same section as figure 15, more highly magnified so that every particle of degenerated myelin of any size could be accurately drawn. A comparison with figure 6 shows at least twice as many large and medium-sized degenerated fibers in the mesencephalic root, but not as many fibers and fine collaterals in the trigeminal motor nucleus, nor among a group of small cells situated median and dorsal to the trigeminal sensory nucleus. × 48.

17 Left half of a transverse section from the same series (ex. 64) as figures 15 and 16, but through the extreme cephalic end of the trigeminal motor nucleus, thirty-one sections further cephalad. The lesion (*Les.*) has severed over one-half of the locus cœruleus fibers at this level, though some distance from the trigeminal motor nucleus. Descending degenerated mesencephalic root fibers appear in two places in this section—in the locus cœruleus between the brachium conjunctivum and the fourth ventricle, and scattered through the trigeminal motor root between the trigeminal sensory and motor nuclei. In the former these fibers make up practically all of the tract; while in the latter they appear to constitute at least two-thirds of the mesencephalic root fibers. × 8.

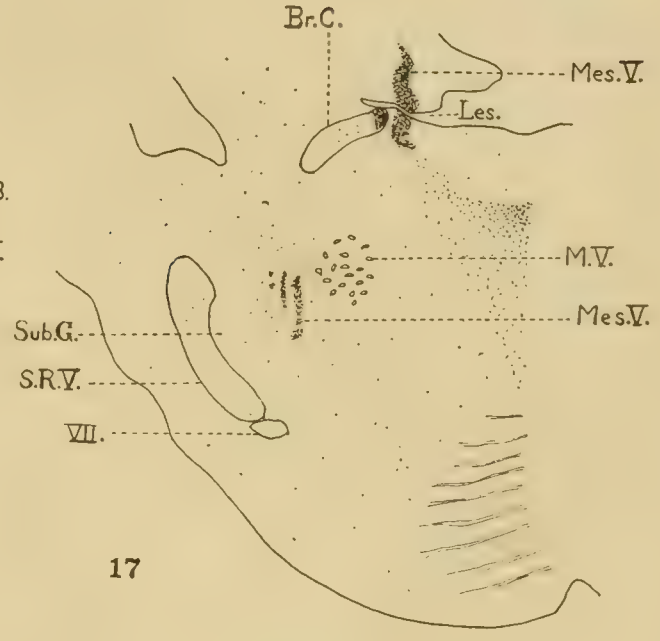

Mes.V.
Mes.C.
IV

13

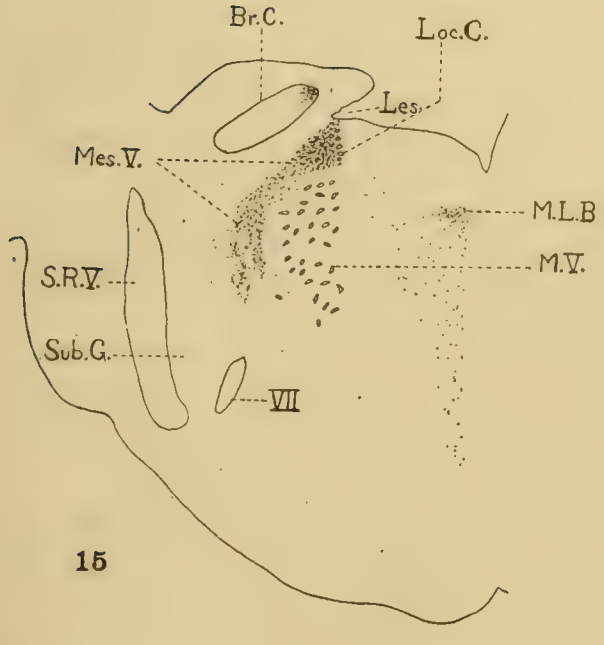

Mes.V.
IV


Br.C.
Loc.C.
Les.
Mes.V.
M.L.B.
Col.
M.V.
S.R.V.
VII

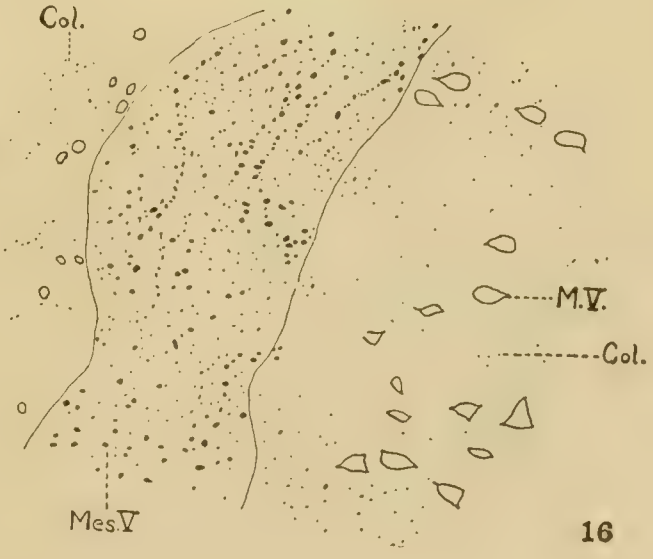
14


Br.C.
Mes.V.
Les.
M.V.
Mes.V.
Sub.G.
S.R.V.
VII.

17


Br.C.
Loc.C.
Les.
Mes.V.
M.L.B
M.V.
S.R.V.
Sub.G.
VII

15


Col.
M.V.
Col.
Mes.V

16

PLATE 4

EXPLANATION OF FIGURES

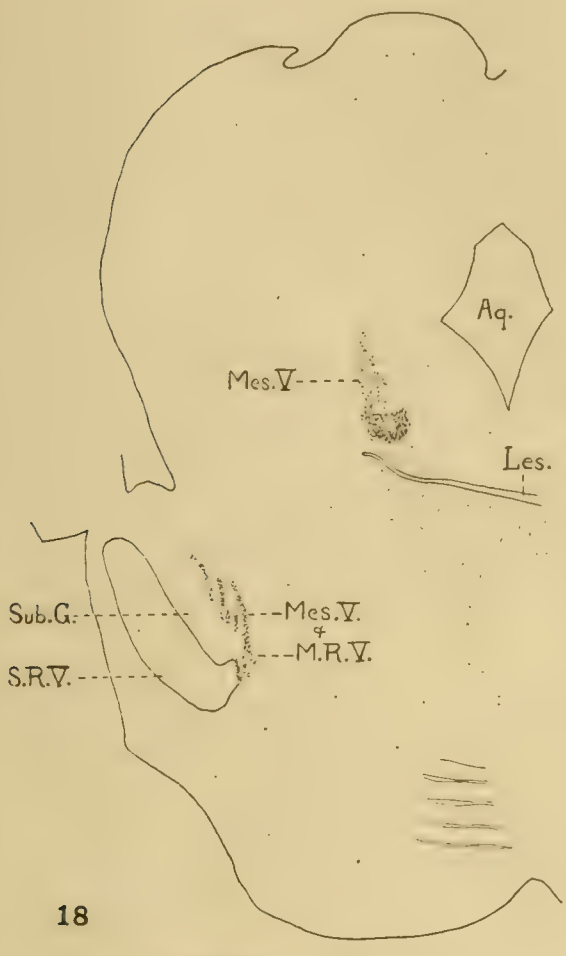
18 Left half of a section twenty-one sections cephalad of figure 17 (ex. 64), passing through the caudal end of the inferior colliculus (corpora quadrigemina). Observe that the lesion (*Les.*) has completely severed the mesencephalic root at this level. As in the previous section, the degenerated descending mesencephalic root fibers appear in two places: 1) opposite the aquæductus cerebri immediately lateral to the central gray, and 2) intermingled with the trigeminal motor fibers, which are approaching the trigeminal sensory root directly median to the trigeminal sensory nucleus (substantia gelatinosa). × 8.

19 Similar view to figure 18, but from a section fifty-two sections cephalad through the cephalic end of the inferior colliculus. This section is above the lesion, so that the few degenerated fibers shown in the mesencephalic root opposite the aquæductus cerebri are ascending fibers having their cell bodies in the semilunar ganglion. The trigeminal motor root, which consists of about one-fifth descending mesencephalic root fibers taking origin from cells in the mesencephalon and locus cœruleus, now occupies a position directly median to the trigeminal sensory root. There is no connective-tissue sheath separating these two roots, but they are sharply marked off, absolutely no intermingling of fibers. See also figures 11, 20, and 28. × 8.

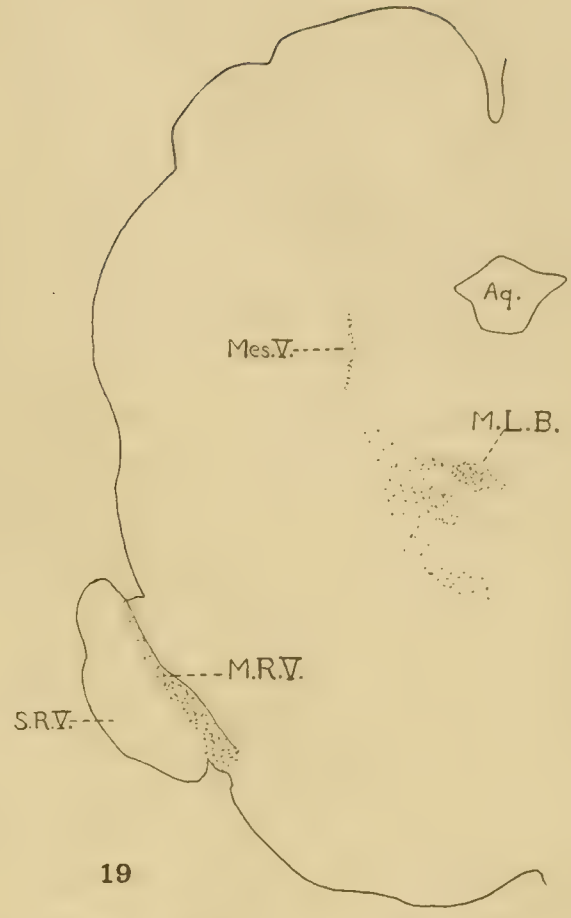
20 Tranverse section through the left trigeminal roots (lesion side) from the same series as figures 14 to 19 (ex. 64), about half way between the exit of the trigeminal motor root and the semilunar ganglion, and sufficiently magnified so that each degenerated medullary sheath of any size could be accurately sketched. Observe that by far the greater part of the trigeminal root is sensory and contains no more degenerated fibers than would be found in a section of a normal root, that the motor root is mainly ventral to the sensory, the line of separation being sharply marked, and about one-fifth of its fibers are degenerated mesencephalic root fibers taking origin from cells in the mesencephalon and locus cœruleus. Note that the trochlear nerve in the sheath surrounding the trigeminal roots contains many degenerated fibers. It was undoubtedly severed in this experiment. × 24.

21 Transverse section through the semilunar ganglion from the same series as the previous figures (ex. 64). Observe the trigeminal motor root, containing degenerated descending mesencephalic root fibers, passing through the ventral portion of the ganglion and separated from the ganglion by a connective-tissue sheath. There are no more degenerated fibers in this ganglion than would occur in any normal ganglion. × 12.

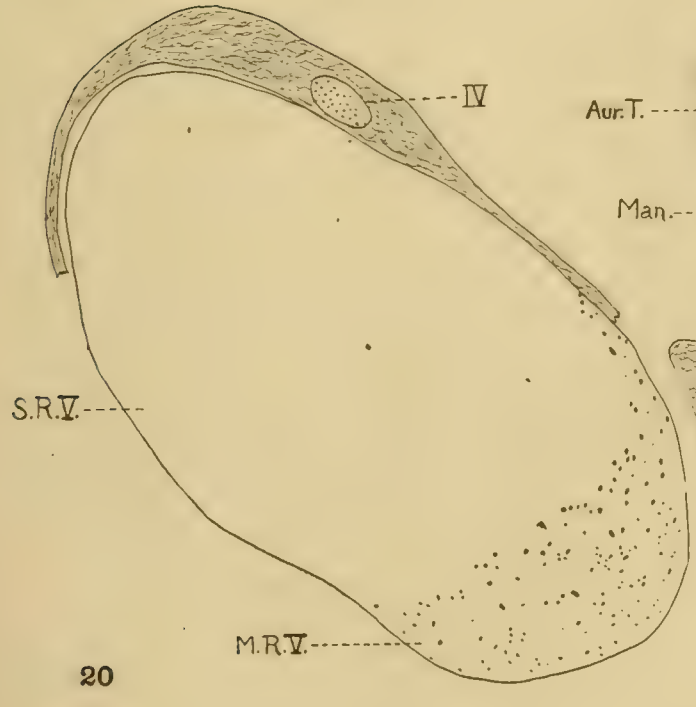
22 Transverse section through the various trigeminal nerves a little peripheral to the semilunar ganglion from the same series as the preceding drawings (ex. 64). To the right will be seen two entirely sensory nerve trunks, ophthalmic and maxillary, in which there are no degenerated descending mesencephalic root fibers. To the left the mandibular nerve will be seen to have separated into three portions: 1) The nervus auriculotemporalis, which is purely sensory and contains no degenerated descending mesencephalic root fibers. 2) The N. mandibularis proper, which with the exception of a few motor fibers for the mylohyoid and digastric muscles is sensory and contains no degenerated descending mesencephalic root fibers. 3) Each of the motor portions of the N. masticatorius, namely, the N. massetericus, N. pterygoideus, and N. temporalis profundus, contains numerous degenerated descending mesencephalic root fibers, while the N. buccinatorius, which is entirely sensory, contains none. × 8.


Aq.
Mes.V.
Les.
Sub.G.
Mes.V.
&
M.R.V.
S.R.V.

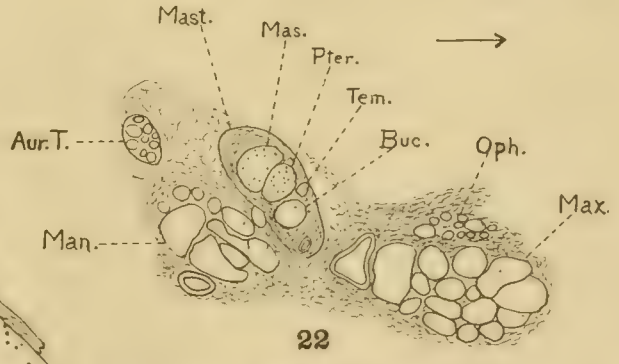
18


Aq.
Mes.V.
M.L.B.
M.R.V.
S.R.V.

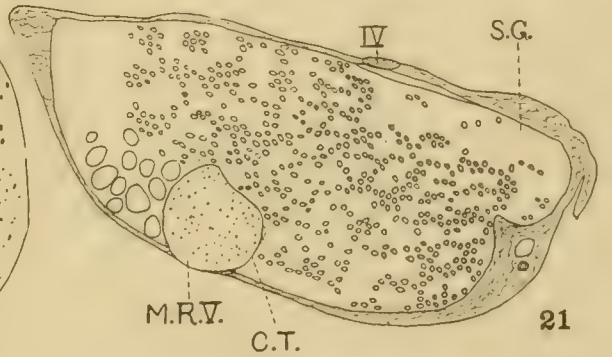
19


IV
S.R.V.
M.R.V.

20


Mast.
Mas.
Pter.
Tem.
Aur.T.
Buc.
Oph.
Max.
Man.

22


IV
S.G.
M.R.V.
C.T.

21

PLATE 5

EXPLANATION OF FIGURES

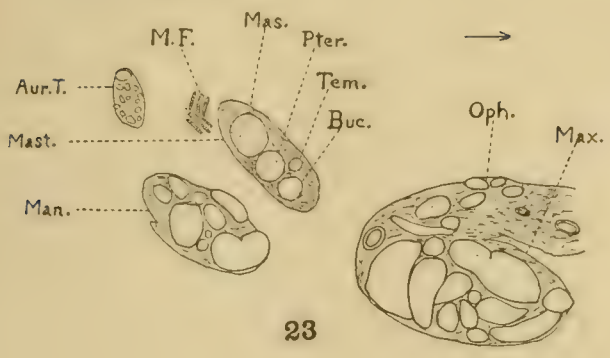
23 Similar view to figure 22 through the trigeminal nerves (ex. 64), but a little more peripheral. Observe that the various nerves are further separated and that the degenerated descending mesencephalic root fibers are confined to the motor components of the nervus masticatorius, namely, the masseter, pterygoid, and deep temporal branches. × 8.

24 From the same section as figure 23, but showing the mandibular, auriculotemporal, and masticator nerves, together with a strand from the ophthalmic and maxillary nerves (a and b) more highly magnified so that almost every particle of degenerated myelin could be accurately drawn. This figure clearly shows that there are no more degenerated fibers in the auriculotemporal, mandibular, and buccal portions of the masticator than there would be in a section of any normal nerve. Also that the degenerated descending mesencephalic root fibers are confined to the masseter, pterygoid, and deep temporal portions of the nervus masticatorius. × 32.

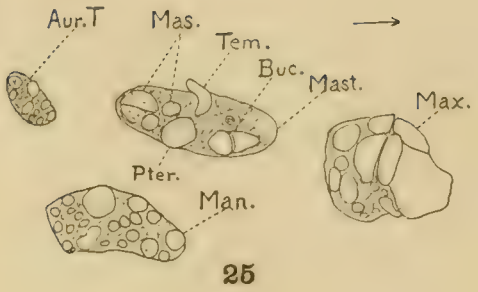
25. Transverse section of the trigeminal nerves from the same series as figure 23 (ex. 64), but more peripheral. The nervus ophthalmicus does not appear in this section and the other nerves have become further separated. As in the previous figures, the degenerated descending mesencephalic root fibers are confined to the motor branches of the N. masticatorius, namely, the masseter, pterygoid, and deep temporal nerves. Observe the latter leaving the main trunk to go to the temporal muscle. This is the most distal of the two deep temporal nerves; the first or most central was given off from the masticator nerve shortly after it left the semilunar ganglion. × 8.

26 As above (ex. 64), but from a more peripheral section. Like the previous sections, the degenerated descending mesencephalic root fibers are confined to the motor portion of the nervus masticatorius, but only two such nerves remain in this trunk, the N. massetericus and N. pterygoideus. The N. temporalis profundus has left this trunk to go to the temporal muscle, the N. buccinatorius, a sensory branch containing no descending mesencephalic root fibers, appears in this section a little lateral to the N. maxillaris. × 8.

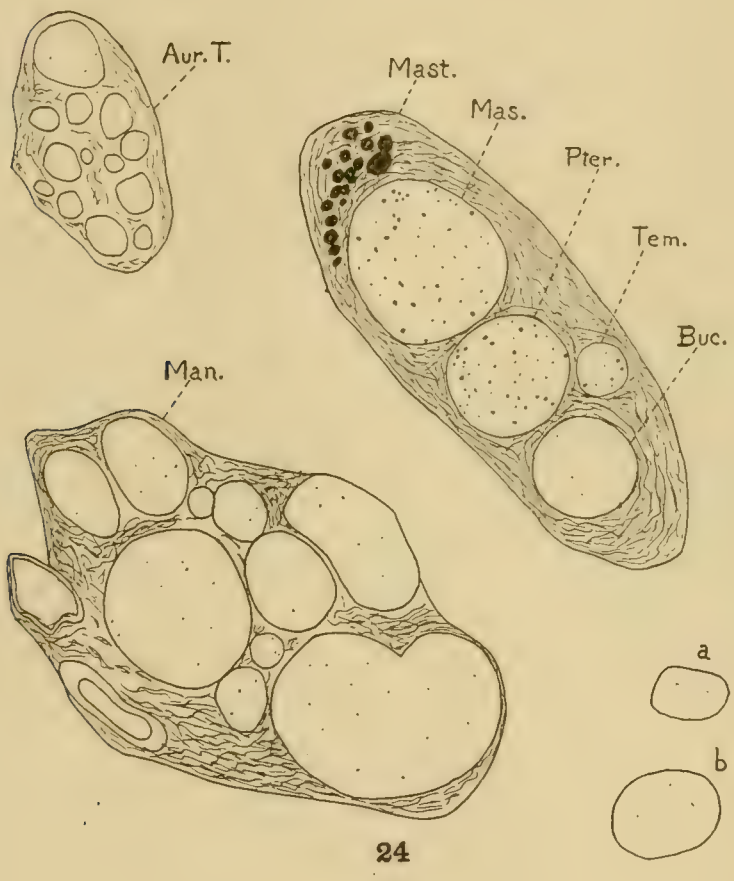
27 Shows the nervus masticatorius from the same section as figure 26 more highly magnified so that every particle of degenerated myelin could be accurately sketched. Observe the degenerated descending mesencephalic root fibers in the masseter nerve and its branch, and in the pterygoid nerve. The pterygoid nerve, which is not branched, is about the same size and exhibits about as many degenerated fibers as it did more centrally in figure 24. On the other hand, the masseter nerve has branched and the main trunk is smaller than it was further centrad, but retains about the same proportion of degenerated to normal fibers. × 32.


M.F.
Mas.
Pter.
Aur.T.
Tem.
Buc.
Mast.
Oph.
Max.
Man.

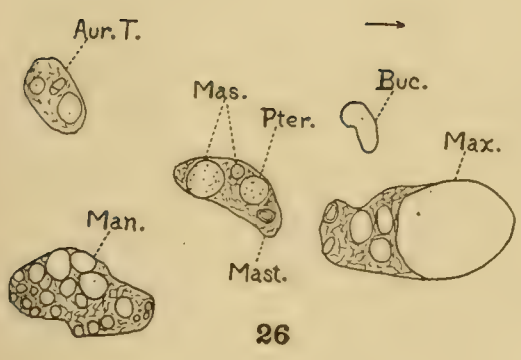
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Aur.T
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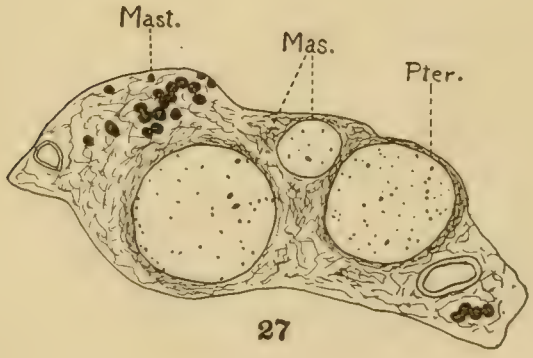
25


Aur.T.
Mast.
Mas.
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Tem.
Buc.
Man.
a
b

24


Aur.T.
Mas.
Pter.
Buc.
Max.
Man.
Mast.

26


Mast.
Mas.
Pter.

27

PLATE 6

EXPLANATION OF FIGURES

28 to 30 are from another series of a guinea-pig's brain stem, including the trigeminal roots, semilunar ganglion and trigeminal nerves. In this experiment (no. 65) the mesencephalic root was severed in the region of the locus cœruleus above the trigeminal motor nucleus, and the incision extended deeper than was intended so that a part of the motor nucleus was injured. This experiment served as a check for determining absolutely which nerves contained motor fibers. The degenerated fibers were stained after a modified Marchi method. It should be noted that the trigeminal roots were bent outward during the process of fixation so that the trigeminal roots and semilunar ganglion appear in somewhat oblique longitudinal section.

28 Approximately longitudinal section through the trigeminal roots, semilunar ganglion and formation of some of the trigeminal nerves (ex. 65). As in the previous series, note the general ventral position of the motor root and its sharp line of demarcation from the sensory root and the semilunar ganglion. Also the presence of many more degenerated medullary sheaths, most of which are trigeminal motor fibers and the balance are descending mesencephalic root fibers. Observe that there are no more degenerated nerves in the sensory root and semilunar ganglion than would occur in a section of any normal semilunar ganglion. Also that no degenerated motor fibers or descending mesencephalic root fibers pass to the semilunar ganglion. × 12.

29 A more peripheral section from the same series as figure 28. At this level all of the components of the mandibular nerve have appeared, but it is not cephalic enough to show the differentiation of the semilunar ganglion into the ophthalmic and maxillary nerves. Observe that there are no more than the normal number of degenerated medullary sheaths in the semilunar ganglion, auriculotemporal and buccal nerves, and that most of the degenerated nerve fibers are found in the masseter, pterygoid, and deep temporal branches of the masticator nerve. They consist of both trigeminal motor fibers and descending mesencephalic root fibers. The few degenerated fibers found in the mandibular nerve proper are trigeminal motor fibers for the mylohyoid and digastric muscles. × 8.

30 Peripheral section of the nervus massetericus and three of its branches, one of which has entered the masseter muscle (ex. 65). This section was sufficiently magnified to allow for the accurate drawing of every particle of degenerated myelin. A comparison with a similar section (ex. 64) shows many more degenerated fibers in the peripheral branches, the increase being trigeminal motor fibers. Also there are as many, if not more, degenerated fibers in the peripheral branches than there were in the main trunk of the masseter nerve, indicating a branching of these fibers. × 32.

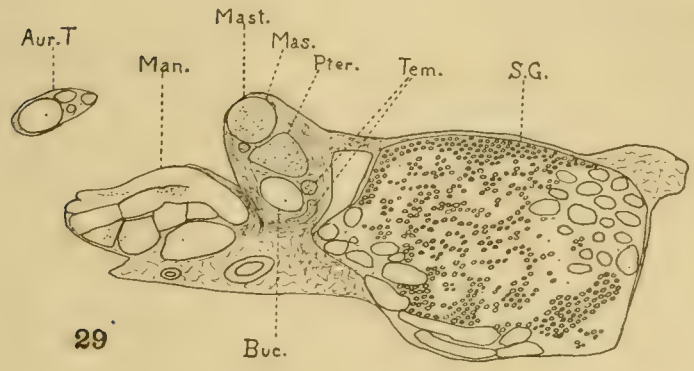
31 to 35 are isolated cells taken from a series of a brain stem and semilunar ganglion of a guinea-pig, stained after a modified Cajal method. All drawings were from the same magnification.

31 (a) One of the semilunar ganglion cells, which is similar in every respect to a typical spinal ganglion cell. (b) A locus cœruleus cell from the same series. Note identical type of unipolar sensory cell, with the exception of the coiled process. × 66.4.

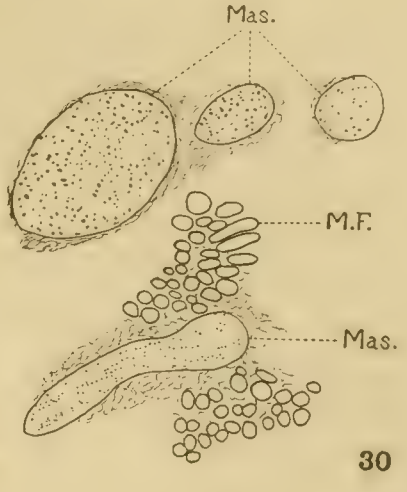
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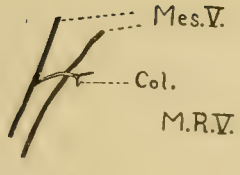
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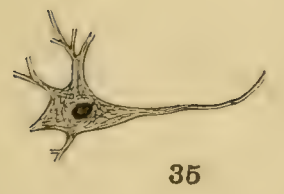


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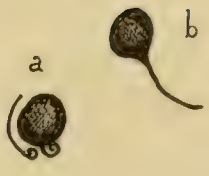


32





35



31



33

32 Two mesencephalic root cells taken from the level of the trochlear nucleus. They are identical with the locus cœruleus cells and differ from the semilunar ganglion cells only by the absence of a coiled process, but are not comparable in any way to the larger trigeminal multipolar motor cells (figs. 33e and 35). × 66.4.

33 (c) and (d) Two types of cells found in the inferior colliculus a little median to the mesencephalic root fibers. The multipolar type of cell is very rare. (e) One of the large trigeminal multipolar motor root cells. × 66.4.

34 Two descending mesencephalic root fibers passing around the trigeminal motor nucleus. From one a collateral is given off to pass toward the trigeminal motor nucleus. × 66.4.

35 Large trigeminalmultipolar motor root cell showing dendrites and axone. × 66.4.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JANUARY 6

CONCERNING REISSNER'S FIBER IN TELEOSTS[1]

HOVEY JORDAN

ONE PLATE (TEN FIGURES) AND TWO TEXT FIGURES

Uncertainty as to the time when I shall be able to resume work on unfinished studies of Reissner's fiber makes it seem best to publish now a short summary of what I have already done and the general conclusions reached, even though the latter may have to be modified as a result of future work.

According to the original plan, the investigation was to have been conducted along four main lines: first, the histology of the fiber and related structures; secondly, the development of the same; thirdly, the degeneration and regeneration of the fiber following operations; fourthly, the function of the fiber. Considerable work has already been done on all of these subjects, but on the last two particularly it is, as yet, incomplete.

Several different methods of study have been employed, but they need not be described here. It is not necessary, either, to go into any considerable account of the previous work on Reissner's fiber, because an extensive résumé will be found in a recent paper by Nicholls.[2]

It may be well, however, to mention the two principal and most recent theories concerning the histological and physiological nature of the fiber. Sargent (1900 to 1904) advocated the view that it is nervous in structure and that functionally it is an 'optic-reflex' apparatus extending, in the lumen of the central nervous system, backward from the region of the posterior commissure of the brain to the posterior limit of the spinal cord.

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, No. 310, and Contributions from the Bermuda Biological Station for Research, No. 100.

[2] Nicholls, G. E. 1917. Some experiments on the nature and function of Reissner's fiber. Jour. Comp. Neur., vol. 27, pp. 117–200, 4 plates, 4 text figures.

He regarded it as being connected at its anterior end chiefly with the optic centers of the brain, at its posterior end with the motor roots of the spinal cord, and, farthest back, with certain so-called 'posterior canal cells,' which are themselves connected to the walls of the cord. In several papers, written either conjointly or individually, Dendy and Nicholls (1902 to 1917), on the contrary, have maintained that the fiber itself is nonnervous; that functionally it is a mechanical apparatus for controlling "automatically the flexure and pose of the body." It is thought to do this by exerting a tension upon the ventricular fibers of certain supposedly sensory cells in the region of the posterior commissure of the brain and in the spinal cord, with which the fiber is believed to be connected.

My own studies have been made chiefly upon two teleosts, the brook-trout (Salvelinus fontinalis) and the hamlet (Epinephelus striatus). I am of the opinion that Reissner's fiber is ependymal in origin and that it does not function as a nervous apparatus either directly, as maintained by Sargent, or indirectly, as argued by Dendy and Nicholls.

In sections of young trout brains, which were purposely cut in a parasagittal plane (figs. 1, 2, A, and B), the fiber is seen to originate (as shown by Sargent) from the region surrounding the posterior commissure. In the trout, however, its cells of origin are all confined (as shown by Nicholls for other forms) to that median strip of columnar epithelium which extends along the anterior face of the region of the posterior commissure from its ventral border nearly to its dorsal extremity, the 'sub-commissural organ' of Dendy and Nicholls. From the superficial end of nearly every cell, probably from all of them, a very fine fibril (*fbrl.'*), which is apparently single, and in the epithelium lies within a sheath, extends, without the sheath, into the lumen of the brain and fuses with those from other cells into somewhat larger fibrillae. These, in turn, converge to a point a little back of the posterior commissure (figs. 1, 2, and B) to constitute the extreme anterior end of Reissner's fiber. These fibers when cut transversely (figs. 2, 3, *fbrl.''*) appear as small dots, each surrounded by a circle—the cross-section of the sheath.

From the deep end of each of these cells a single, nearly homogeneous, highly refractive process (*fbrl.*) extends to the membrana limitans externa of the brain, with which it fuses (figs. 1 to 5 and B). So far as I have been able to determine, none of these cells give rise to processes which turn into the nerve tracts of the posterior commissure. The cells usually are arranged somewhat as in a typical columnar epithelium (fig. 1), but in the later stages their axes become more and more oblique to the inner surface of the brain wall whence the fibrillae emerge (fig. 3). The connections of the processes, however, are not altered.



Fig. A General view of sagittal section of brain, to show the region of the posterior commissure and also a probable migration, from the roof of the brain, of cells concerned in the formation of Reissner's fiber. The cells believed to be migrating have been projected on the plane of the section from another individual than that which furnished the outline. 50 days.

By making thick (30 μ to 40 μ) frozen sections of the brains of young trout, it was possible under a lens to isolate the region of the posterior commissure and the epithelial wall covering it. Such isolated regions, when properly treated with dissociation reagents, gave good 'isolation preparations' of the epithelial cells now under consideration. These cells are larger than the typical nerve cells. All of them seem to possess a characteristic structure. The deep end of the cell body is produced into the process already mentioned, which extends to the external limit-

ing membrane. The middle portion of the cell body is spindle shaped and its superficial end is prolonged, as has been stated, into a process which reaches to the lumen of the brain. This process is differentiated into an outer, less refractive portion, the sheath, and a very fine, highly refractive axial fibril (*fbrl.'*). It is this fibril which projects into the lumen and contributes to the

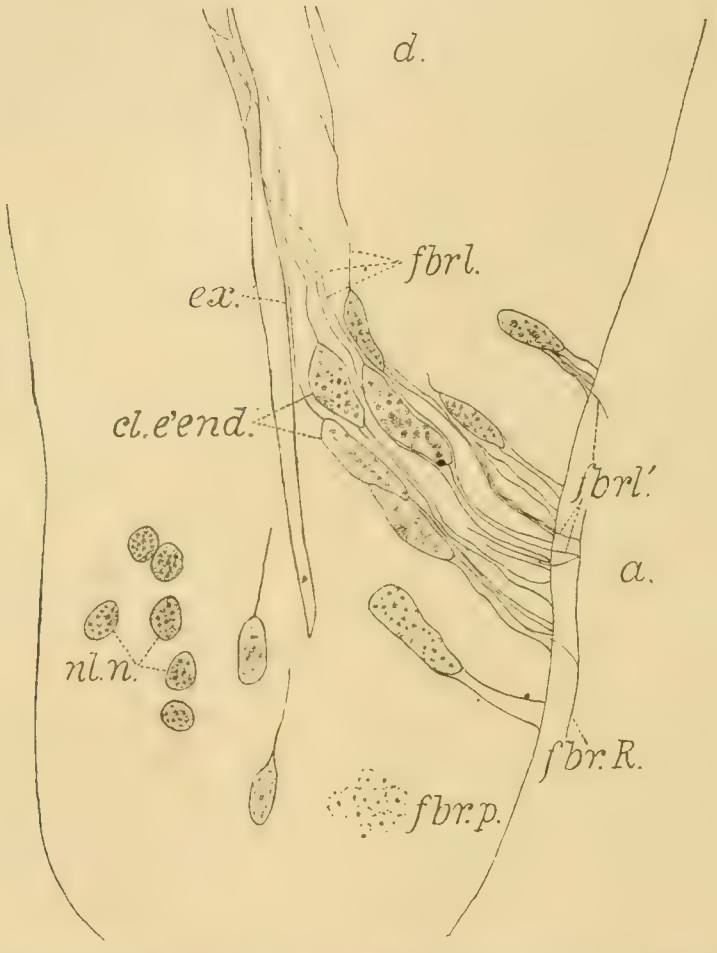


Fig. B. Parasagittal section, to show ependymal cells of the sub-commissural organ and the connections of their fibrils. (Compare with figure 3.) 100 days. × 750.

formation of Reissner's fiber. Its deep end can be traced as far as the nucleus of the cell, but, so far as I can discern, not beyond the nucleus (figs. B and 5).

It is to be seen in sagittal sections, where Reissner's fiber can be traced throughout the entire length of the spinal cord, that it extends continuously from its origin in the brain to its insertion

in the region of the sinus terminalis. It is a very fine (0.4 μ in diameter) taut thread, lying in the lumen and, usually, somewhat nearer the ventral than the dorsal wall of the central canal. The exact relation of the fiber to the cells which constitute the walls of the spinal cord is often obscured by what appears to be a coagulum. However, by a partially successful process, aimed at removing the fluid of the central canal before fixation, and by then holding the fishes in a vertical position during fixation, it was often possible to obtain longitudinal sections in the median plane of the cord which were fairly satisfactory in this respect.

Many of the ependymal cells, particularly in the roof of the cord, possess a structure which is somewhat similar to that of the cells in the 'sub-commissural organ.' Some of them send a fibril into the lumen of the cord. This fibril varies in length in different cells. In some cases it does not extend to Reissner's fiber, while in others it passes beyond. Sometimes, also, there seems, at first sight, to be a connection between the two. According to my observations, however, none of these fibrils has a structural union with Reissner's fiber, which seems, in the stages I have studied, to be free from the walls of the cord throughout its whole length (figs. 6 and 7).

The posterior end of the spinal cord, the region of the sinus terminalis, is not closed by the cells of the neural wall, and Reissner's fiber, often somewhat enlarged, passes through the opening thus left to end in a 'terminal plug,' slightly posterior to the end of the neural tube. This plug is in close connection, probably in continuity, with the connective tissue which surrounds the spinal cord in this region (figs. 8 to 10). It may be that the fiber here also has some histological connection with the membrana externa of the neural tube.

In its staining properties the fiber and its related structures resemble most closely neuroglia and connective tissues. Although a wholly satisfactory selective stain for the fiber is yet to be found, it may be said that the fiber, as well as the fibrils from the ependymal cells and also the brain membranes, all stain characteristically and strongly in dyes which are supposedly specific for elastin (e.g., in resorcin-fuchsin). In rather unsatis-

factory silver preparations (Bielschowsky, Ranson) the fiber and its cells of origin remained unstained.

In the brook-trout the beginning of the development of the 'sub-commissural organ,' and likewise of Reissner's fiber, is nearly contemporaneous with the appearance of the first fibers of the posterior commissure. As seen in parasagittal sections taken near the median plane (fig. A), the organ is at first a small region of slightly thickened epithelium, which, with the enlargement of the posterior commissure, protrudes more and more into the brain lumen from the anterior portion of the roof of the midbrain. With further development and the addition of more commissural fibers the protrusion becomes more prominent and at length has the appearance of a fold of the epithelium, the deep surfaces of the two layers constituting the fold being separated by only a thin layer of connective tissue. In sagittal sections it now has the form of a tongue-like projection extending into the lumen of the brain and making an angle of about 45° with the long axis of the neural tube. At a later stage this tongue-like region is more and more elongated in a caudal direction, and the angle made by its axis with that of the neural tube decreases (figs. 1 to 3).

The histological character of the 'sub-commissural organ' is established at an early stage (twenty days), when probably every one of the ependymal cells sends a fibril into the brain ventricle. The number of these cells increases somewhat as development proceeds, but it is fairly constant from about the fiftieth day after fertilization, there being approximately fifty to sixty cells in a single sagittal section 5 μ thick. After entering the lumen, most of the fibrillar processes from these cells converge into larger fibrils. At about this time several cells, which are probably ependymal, detaching themselves from the roof of the brain, enter the lumen (fig. A, *cl. R.*) and become elongated in an anteroposterior direction. Here they seem to fuse gradually and completely with the anterior portion of Reissner's fiber (fig. 1). This detachment of ependymal cells from the roof of the brain appears to be followed by a similar activity in the wall

of the spinal cord throughout its whole length.[3] Some of the stages in this process are shown in figures 6 to 9. In this particular case, however, it is difficult to say whether these cells are producing or regenerating the fiber. The fiber is fully formed in trout embryos which are about half an inch in length (approximately fifty days old).

Until recently very little work has been done on the regeneration of Reissner's fiber. In this connection Sargent, first, and afterwards Nicholls, showed that when the fiber is experimentally severed it retracts toward the place of its attachment. Nicholls has also stated that the fiber regenerates after such severance, and also that in its recoil it takes on a spiral form, as was also held by Sargent. He believes that the fiber is a living protoplasmic structure, having inherent ability to uncoil, to resume approximately its original form, and to establish new connections with the 'terminal plug.' My own observations on this subject are, as yet, very few, but I can confirm the statement that the fiber recoils spirally when it is experimentally severed. I have a few sections, too, which may be taken to suggest a subsequent straightening of the fiber, although they do not prove it, nor do they suggest the method by which the straightening is effected. This process may be purely mechanical and not the result of any inherent vitality, or it may even be brought about chiefly through the agency of cells which enter the lumen of the cord from the wall and seem to fuse completely with the fiber (figs. 7 to 9).

There has been much controversy concerning the function of Reissner's fiber. Sargent's theory that it is a nerve-fiber tract

[3] In embryos which are about twenty-five days old there is a considerable migration of cells from many portions of the brain wall into the lumen In addition to the cells which form Reissner's fiber, there are blood corpuscles and peculiar cells, particularly from the regions near the velum transversum, which, entering the cavity of the brain, seem to disintegrate into a substance indistinguishable microscopically from the coagulated cerebrospinal fluid. These cells probably form an important constituent of the complex brain fluid. This migration decreases with further development, but it has not been determined yet whether it is repeated at later stages.

serving as an 'optic-reflex' transmission path is denied by Nicholls, who adopts the theory, originally suggested by Dendy, that the fiber automatically controls the flexure of the body.

In support of this idea Nicholls has recently brought forward some experimental evidence. He pierced the tails of a large number of sharks and rays in the region of the sinus terminalis, his purpose being to sever the posterior connections of Reissner's fiber. This experiment usually caused the fish to assume an abnormal attitude, in most cases an uptilting of the posterior end of the body. Subsequent microscopic examination of the tails of these fishes revealed the fact that the reaction occurred, in general, only when Reissner's fiber had suffered considerable recoil.

I have recently repeated on the brook-trout these experiments, and have performed several others of a slightly different nature. The results do not seem to confirm for this teleost Nicholls' theory, for, although a mutilation of the sinus terminalis region does cause the reaction which he described, the same reaction can also be induced by cutting the tail in several other regions which, being outside the limits of the posterior end of the spinal cord, leave the sinus terminalis, and hence Reissner's fiber, intact. Whether this reaction is due to some purely mechanical effect or is brought about through the agency of the nervous system, I have not yet determined. There is some evidence to show that the normal attitude of the body is reassumed as soon as the tail has healed and regenerated.

Operations aimed at cutting the fiber in the fourth ventricle were carried out on the hamlet (Epinephelus striatus Bloch) at Bermuda. The reactions of operated and of normal fishes to all the various categories of stimuli applied were practically identical. In view of the fact, however, that microscopic examination of the operated brains failed to establish conclusively the success of the operation, it is not desirable to give much weight to these experiments. They merely suggest that the fiber may have no function so far as concerns the reactions of the fish to a variety of stimuli. It was my intention to have repeated these

experiments during the present (1917) summer, but that has proved to be impossible and must be deferred.

The fiber was also cut near the posterior end of the cord in a few fishes. In these cases the reactions apparently remained normal.

It seems to me that the evidence which I have thus far secured points toward the conclusion that Reissner's fiber is ependymal in origin and that it plays no important part in the reactions of the fish. It is necessary, however, to complete all of the proposed work, which I hope soon to do, before this question can be answered with complete satisfaction.

Cambridge, September, 1917.

PLATE 1

EXPLANATION OF FIGURES

1 Parasagittal section of sub-commissural organ. 49 days. × 325.

2 Parasagittal section of sub-commissural organ. 60 days. × 400.

3 Parasagittal section of sub-commissural organ. 100 days. × 325.

4 and 5 Single ependymal cells obtained by dissociation. The diameter of the fibrillar process apparently depends on its length. × 1608.

6 A somewhat diagrammatic view of a sagittal section of the spinal cord (neural tube) near the middle of the body. A connection between Reissner's fiber and the fibrils of ependymal cells is simulated. The cell in the lumen of the tube is helping to form, or to repair (probably the latter) Reissner's fiber. 60 days. × 750.

7 Sagittal section of spinal cord near the middle of the body, showing Reissner's fiber apparently free from the walls of the neural tube. The dorsal wall is not shown in its whole thickness. 100 days. × 750.

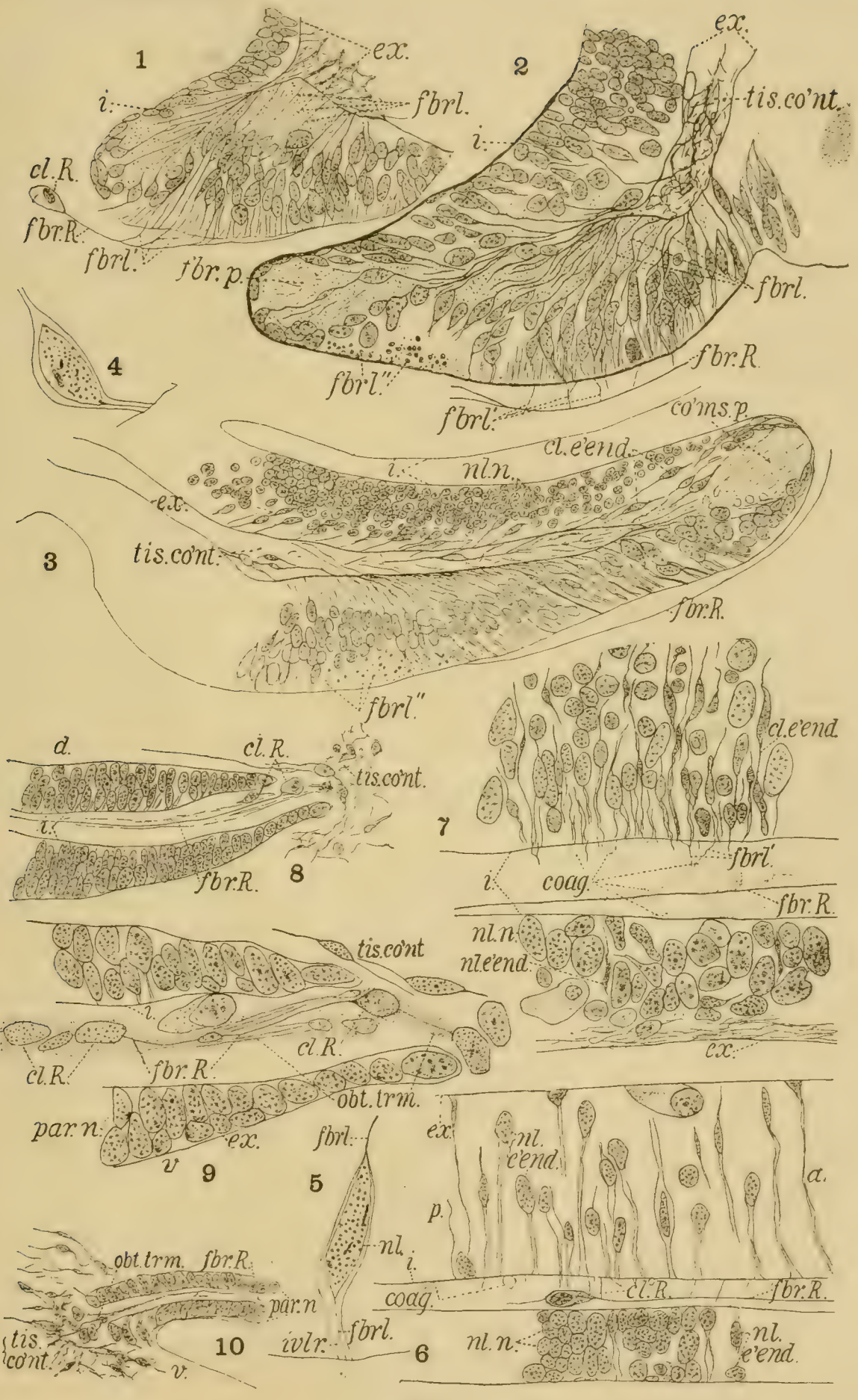
8 Sagittal section in the region of the sinus terminalis. × 325.

9 Posterior end of the section shown in figure 8, at a slightly different focus and more highly magnified. Several cells are seen to be in process of metamorphosing into the posterior end of Reissner's fiber; others, apparently, are just entering the lumen of the neural tube. × 750.

10. Sagittal section through the sinus terminalis, showing the terminal plug. There is only one cell in process of metamorphosing into the Reissner fiber, and this is, apparently, in continuity with the connective tissue lying beyond the posterior end of the neural tube. × 325.

ABBREVIATIONS

a, anterior
cl. e'end., ependymal cell
cl. R., cells in process of forming Reissner's fiber
coag., coagulum
co'ms. p., posterior brain commissure
d., dorsal
ex., external limiting membrane
fbr. p., fibers of posterior commissure
fbr. R., Reissner's fiber
fbrl., fibrils from deep (proximal) ends of ependymal cells
fbrl.' fibrils from superficial (distal) ends of ependymal cells
fbrl.'', cross-sections of *fbrl.'*
i., internal limiting membrane
ivlr., sheath of *fbrl.'*
nl. e'end., ependymal nucleus
nl., nucleus
nl. n., nucleus of nerve cell
obt. trm., terminal plug of neural tube
p., posterior
par. n., wall of neural tube
sb-co'ms., sub-commissural organ
tis. co'nt., connective tissue
v., ventral


1
ex.
i.
fbrl.
cl.R.
fbr.R.
fbrl'.
fbr.p.
2
ex.
tis.co'nt.
i.
fbrl.
fbr.R.
fbrl''.
fbrl'.
4
co'ms.p.
cl.e'end.
i.
nl.n.
ex.
3
tis.co'nt.
fbr.R.
fbrl''.
d.
cl.R.
tis.co'nt.
i.
fbr.R.
8
7
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fbr.R.
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e'end.

Resumido por el autor, Robert Sidney Ellis.

Estudio preliminar cuantitativo de las células de Purkinje en el cerebelo normal, subnormal y senil del hombre, con algunas notas sobre la localización funcional.

En el presente trabajo se compara el número de células de Purkinje en el cerebelo normal, subnormal y senil del hombre. El autor ha hecho correcciones para las diferencias de volumen de los cerebelos estudiados, de modo que los valores consignados representen el número de células existentes en áreas equivalentes, tomadas como unidad. Ha contado las células de seis áreas de cada mitad del cerebelo, determinando de este modo el número de células de Purkinje que existen normalmente en áreas equivalentes del cerebelo de tamaño normal. Comparando con la norma asi establecida ha podido comprobar que el número de células de Purkinje es muy deficiente en el cerebelo de los idiotas e imbéciles. Del mismo modo, en el cerebelo del viejo se observa también una disminución progresiva en el número de dichas células. Existe cierta correlación entre estas deficiencias celulares y las deficiencias en la coordinación motríz.

Translation by Dr. José F. Nonidez.
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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JANUARY 6

A PRELIMINARY QUANTITATIVE STUDY OF THE PURKINJE CELLS IN NORMAL, SUBNORMAL, AND SENESCENT HUMAN CEREBELLA, WITH SOME NOTES ON FUNCTIONAL LOCALIZATION

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TWO FIGURES AND ONE CHART

INTRODUCTION[1]

In the spring of 1916, while examining the cerebellum of a general paralytic, the writer was first impressed by the fact, familiar perhaps to most neuropathologists, that in this disease there is often a disintegration and disappearance of a large number of the Purkinje cells, leaving, however, the basket of fibers which normally surrounds them. Over a year later, while examining the cerebellum of a microcephalic idiot, the same scarcity of Purkinje cells was observed, with the difference, however, that the section did not show the same evidence of the cells having become reduced in number by disintegration; the empty pericellular baskets were not found as in the case of paresis; it seemed, rather, that through some defect of development the normal number had never been present.

This difference presented an interesting problem and it seemed worth while to make a more careful quantitative study of the Purkinje cells in different types of cerebella. In order to get

[1] The writer is indebted to the staffs of the Vineland (N. J.) Training School and of St. Vincent's Hospital (Philadelphia) and to several physicians in other hospitals for assistance in securing the material used in this study. He is also indebted to the staff of the Wistar Institute, especially Drs. Greenman, Donaldson, and Hatai, for their encouragement and assistance in numerous ways during the course of the investigation: to all of these he wishes to express his appreciation.

a fair basis for comparison, a number of cerebella were studied and the relative frequencies of cells noted. In some of the cases the cells appeared to be almost uniformly distributed and with few large spaces between them; others showed losses similar to the two cases already mentioned.

Among the cerebella examined was one of a man who had died at about the age of sixty-five years after a protracted illness, and this, too, showed a distinct loss of cells. So from this preliminary set of observations it seemed clear that the number of Purkinje cells is variable under different conditions.

It is well known that in paresis, in extreme old age, and in low grades of feeble-mindedness there is ordinarily a considerable degree of deficiency in motor coördination. The question consequently arose, how far is it possible to find differences in the number of cells that will account, partially at least, for the observed differences in behavior?

The writer's primary interest at the time of taking up this investigation lay in the question of the anatomical basis of mental defect, and it seemed not improbable that a careful study of the Purkinje cells might throw some light on one of the most evident deficiencies found in such cases. The human motor mechanism is much more highly developed than that of lower forms, especially with reference to speech, hand movement, and the maintenance of equilibrium while standing or walking. Mental defectives generally show less motor control along these lines, and it is desirable that we know as far as possible the neural basis for such lack of coördination.

A further reason for making a study of the cerebellum in such cases is found in the fact that a number of writers, especially Tredgold ('03) and Bolton ('03, '10) in England, have emphasized, perhaps unduly, the importance of the frontal lobe of the cerebral cortex as the area particularly affected in amentia. It accordingly seemed worth while to determine whether the brains of aments show defects in other parts, such as the cerebellum, which is not generally associated with intelligent reactions as such.

In order to determine the nature and extent of the variations in the Purkinje cells, and especially to determine the differences between normal and subnormal cerebella, a careful study of the numerical distribution of these cells has been made, the results of which it is the purpose of this paper to present.

MATERIAL

For the best results in studying the present problem it would be desirable to have cerebella in perfectly normal condition to be used as the standards with which the subnormal ones are compared. These normal cerebella should be from individuals in the prime of life, who were known to have had good physique and good motor control and who had died either from accident or from some acute disease which did not cause a disintegration of nervous elements. Pathological material is to be had in abundance, but to secure any number of approximately normal cases is next to impossible. In the present study, consequently, it has been necessary to use as our norms cerebella from the museum collection of The Wistar Institute. No claim is made that this material is ideal; it is hoped, however, that sufficient care and caution have been used, both in the procedure followed and in the interpretation of results, to prevent serious errors arising from the material employed.

To obtain norms, the cerebella of three adult whites and of five adult negroes, all males (thirty to forty-two years of age), were sectioned and a preliminary study was made to determine whether or not they had suffered any loss of Purkinje cells. In nearly every case there was at least some maceration and some loss of these cells, but four of the number, three negroes and one white, showed very slight losses, and these were accordingly used as the material for determining the norm. These are the cases listed in table 1. The other four cases were rejected because microscopical examination clearly showed that cells had disintegrated and dropped out, and it would consequently have been impossible to determine from them the number of cells normally present.

Little or no information of importance regarding the clinical histories of these cases was available. They were simply cases that had come to autopsy and their brains had been preserved as a routine measure. Even so, however, the results from the four should give us a close approximation to the conditions present in the average cerebellum as it appears in the hospital population—a group probably somewhat below the average for the community at large in the development of the nervous system.

Nine cerebella from mental defectives, who had died at the Vineland Training School, were selected from the collection in The Wistar Institute Museum and a study was made of each case. Several of these cases have been described by Goddard (Feeble-mindedness, New York, 1914), and his case numbers, preceded by 'G,' enclosed in parentheses, are given after the serial numbers which these brains have on the records of The Wistar Institute. The histories of these defectives, all whites, and listed in table 2 and table 4, are as follows:

14880, male, age 23 years; alcoholism and insanity in family; three miscarriages before the birth of this child; he had had convulsions, marasmus at one year, measles at four years; small in size, head small; poor motor power, poor gait, poor speech; no will power; right handed; fair memory.

15144, male, age 34 years; height, 6 ft. 3 in.; brain weight 1619 grams; supposed insanity and feeble-mindedness on father's side; no deaths in infancy in family; had whooping-cough, measles, and pneumonia; walked at three years; head, bullet-shaped; right side not developed; dragged feet, could thread needles and tie shoes; talked little; could not count.

15145, male, age 24 years; brain weight 1491 grams; normal (?) heredity; first child dead; had measles and whooping-cough; epileptic; abscess on right hip; weak heart; normal electric reaction; grip, rt. 0–lt. 0.

15214, male, age 26 years; brain weight 1065 grams; father alcoholic; five children died young; first child a 'lunatic,' followed by two miscarriages; two other children mentally defective; this, the sixth child; probably congenitally luetic; spasms at nine months; walked at three years; talked at four years; had measles and whooping-cough, abscesses, scrofula, Pott's disease; hunchback, T.B. of vertebrae; weak heart; was apparently normal until nine months; not strong enough to work; grip, rt. 18–lt. 22; could dress self; learns quickly, forgets soon. This case is presented by itself in table 4.

15250 (G. 285), male, age 43 years, mental age three; nervous family; epileptic, had measles and whooping-cough, brain fever at two years; side of face deformed from atrophy of jaw; stooped shoulders; left arch fallen; irregular heart; poor vision, eye fatigue and headache; no abnormal movements; exaggerated reflexes; grip, rt. 21–lt. 23.

15297 (G. 203), male, age 26 years, mental age eight, apparently normal heredity; two deaths in infancy and two other children feeble-minded; physicians believed defect due to congenital lues; did barn and garden work, grip, rt. 33—lt. 31.

15299 (G. 195), male, age 36 years, mental age two; brain weight 335 grams; father probably feeble-minded; this case the first of seven conceptions; two later ones being miscarriages; had whooping-cough and scarlet fever; large head, left scapula higher than right, left testicle undescended; fallen arches; knock-kneed; did no work; grip, rt. 15–lt. 17; could not talk, but would swear freely.

15310 (G. 258), male, age 18 years, mental age four; brain weight 1051 grams; ancestry possibly neuropathic; this case Mongolian in type; Wassermann positive; knock-kneed and stoop-shouldered; died of T.B. after extreme wasting away; left testicle undescended; normal reflexes; ate well and played well; left-handed; grip about .18 for each hand; did not alternate feet in climbing stairs; speech poor; subject to headaches.

15320 (G. 327), female, age 20 years, mental age one; mentality of ancestry doubtful; had large tumor in anterior part of the right hemisphere of the cerebellum; began to grow very weak at eighteen years; walking became poor, and a year later had difficulty in swallowing; helpless in both hips and hands, left hand especially weak; pupil reflexes lost, other reflexes exaggerated.

Subnormal infants. For purposes of comparison, the cerebella of six infants, four negroes and two whites, were studied. These cases are of the type found in charity hospitals where nearly all are below normal and where a very large percentage are illegitimate. Out of twenty such cases, where the brain weights were taken, only two brains weighed as much as the average for their age. Consequently, though the point could not be determined individually, we are safe in assuming that they represent a distinctly subnormal group of the population.

Senescents. The cerebella of five cases of senescence were studied. Enough is known of each of these to make it fairly certain that the changes observed were due primarily to old age rather than to other causes. The age, color, and sex of these are given in table 5, which shows the results of the cell counts.

Paresis. While making this study, the cerebellum from a

case of paresis was sectioned before the cause of death was known, and it seemed desirable to include it as an example of what may happen in this disease. It is from a white man who was of average physique and who died at the age of thirty-three years. The data are given in table 6.

In the course of this investigation careful counts have been made on parts of about forty cerebella and sections from about twenty-five others have been less carefully examined. The twenty-five cases on which the figures given in this paper are based are believed to be typical of the classes under which they are listed.

PREPARATION OF MATERIAL

The cerebellum was removed from these brains, weighed, and the weight in grams recorded. In cases where the specific gravity had changed materially from being for several years in alcohol, the specific gravity was determined and the brain weight was corrected to the normal weight for a cerebellum of that volume. No change of consequence was observed in the specific gravity of those cerebella which had been preserved in formalin. Consequently, no corrections were made on the weights as taken.

After weighing, three blocks were taken from each cerebellum. The entire vermis was removed and cut so that a sagittal section could be made of it entire. Then blocks were cut which would give sections nearly through the middle of each hemisphere and at right angles to practically all the folia. The plane of these was so selected as to cut the lobus biventer, or paramedianus (Bolk), on the under surface, to pass through the dentate nucleus, and to cut the anterior dorsal edge of the hemisphere near the vermis. A section so made shows for comparative purposes the areas of each hemisphere to which different functions have been assigned by Bolk ('05), Rynberk ('07, '12), et al.

Figure 1 shows the plane in which the sections were taken, and figure 2 the appearance of such a section and gives the localization pattern advanced by the above writers.

The blocks of hardened tissue were cut about 5 mm. thick. Their maximum length and maximum breadth were then meas-

ured in millimeters and the measurements recorded on the cards with the weights.

The procedure in dehydrating and embedding the blocks from material preserved in 10 per cent formalin was as follows:

Alcohol 50 per cent + Formalin 6 per cent	2 hours
Alcohol 70 per cent + Formalin 4 per cent	15 hours
Alcohol 90 per cent + Formalin 2 per cent	8 hours
Alcohol 97 per cent	24 hours
Alcohol + Ether, equal parts	24 hours
Parlodion, 2 to 3 per cent	2 to 4 days
Chloroform	6 hours
Benzole	1 hour
Benzole-paraffine	18 hours at 40°C.
Paraffine	3 to 6 hours at 52–58°C.

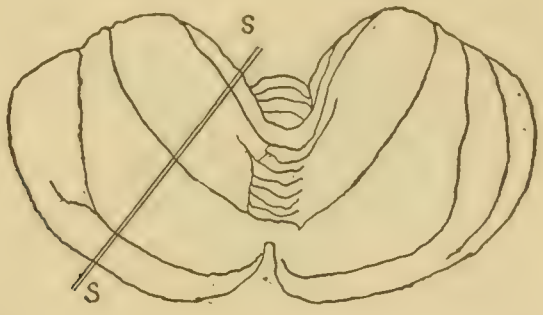


Fig. 1 Human cerebellum seen from above. The line *S.S.* marks the plane of the section which is shown in figure 2.

In the experience of the writer, old formalin cerebella tend to macerate easily, and the molecular layer especially shows a tendency to break away from the internal granular layer. Where this occurs there is always the possibility that some of the Purkinje cells will drop out during the process of cutting and staining. When the above procedure is followed, however, this maceration is not usually found and good preparations are secured. The common practice of washing formalin material in water before dehydrating makes the molecular layer more likely to break away from the rest of the cerebellum, and it should consequently be avoided. Material preserved in alcohol was treated as above with the exception that the blocks were put at once in 90 per cent alcohol without formol.

After embedding, the sections were cut on a rotary microtome at 25 μ, fixed to the slide with Meyer's albumen, and stained with carbol-thionine and eosin. Better results are sometimes secured with the cerebella of infants if Delafield's haematoxylin and Orange G are used instead. Care must be taken that the sections be not treated with absolute alcohol, as this dissolves the parlodion. Equal parts of absolute alcohol and chloroform may, however, be safely used (King, '10).

The most satisfactory fiber preparations from old formol material were secured by Bielschowsky's pyradine method. With a modification of this the pericellular basket fibers have been clearly shown in cerebella that had been in formol for thirteen

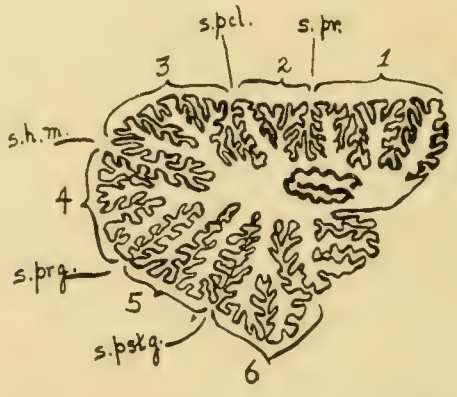


Fig. 2 Section through a hemisphere of the human cerebellum in the plane shown in figure 1. The designations are explained on page 237.

years. If the pyradine is not thoroughly washed out before putting into silver, and a weak solution of silver nitrate, 0.5 to 0.75 per cent, is used and changed frequently, the fibers will be impregnated with silver in one or two days. The sections are then treated as usual. These fiber preparations give valuable assistance in determining the character and causes of the deficiency in cells.

After staining, the sections on the slide were measured and the measurements recorded on the cards with the original measurements of the blocks as cut from the cerebellum at the time of weighing. From these two measurements the percentage of shrinkage is calculated. This will be referred to later.

METHOD OF STUDY

The purpose of this study, as has been stated above, was to compare the number of Purkinje cells in different types of brains, and also to find what numerical differences, if any, exist between different areas of the same cerebellum. Reasoning by analogy from what is known of the cell losses in the cerebrum, it seemed not improbable that some areas might be found more subject to degeneration than others, and, in view of the localization theory of Bolk, it appeared all the more desirable to make a comparison of the different areas to which different functions have been credited. For this purpose each hemisphere has been divided into six areas,[2] as shown in figure 2.

1. Lobus anterior (Bolk); head area; the region anterior to the sulcus primarius (*S. pr.*)

2. Lobulus simplex (Bolk); neck area; from the sulcus primarius to the sulcus postclivalis (*S. pcl.*)

3. Lobulus semilunaris superior; arm area; from the sulcus postclivalis to the sulcus horizontalis magnus (*S.h.m.*)

4. Lobulus semilunaris inferior; arm or leg area; from the sulcus horizontalis magnus to the sulcus pregracilis (*S. prg.*)

5. Lobulus gracilis; leg area; from the sulcus pregracilis to the sulcus postgracilis (*S. pstg.*)

6. Lobulus biventer; leg area (?); part posterior to the sulcus postgracilis, exclusive of the amygdala.

In order to secure exact and strictly comparable measurements of the relative numbers of cells in these different areas and of the relative numbers in the same areas of different cerebella, the following method has been used:

The sections were projected at a magnification of 30 diameters by means of the Edinger projectoscope, and tracings made in pencil of the line of Purkinje cells to be counted. The length of this line as traced was measured in millimeters by means of a map measurer. This divided by 30 gave the actual length of

[2] The first two designations (1–2) are given according to Bolk '05 while the remainder (3–6) are those used in Quain's Elements of Anatomy, vol. 3, Neurology, part 1, eleventh edition, 1908.

the line on the slide. The number of cells in the line was then counted under the microscope, only those cells showing the nucleolus being counted, with the exception that when the cells were so disintegrated that the nucleolus would not appear in the preparation, the cells were counted if they appeared to belong properly to the section. Dividing this total by the length of the line in millimeters gives the number of cells in a line 1 mm. long on the slide, which, as has been stated, is for a section 25 μ thick. To correct this for shrinkage during dehydration and embedding, the value thus obtained for 1 mm. on the slide has been multiplied by the square of the percentage obtained by dividing the sum of the length and breadth of the section on the slide by the sum of the length and breadth of the block as first cut from the cerebellum. This gives the number of cells found in a line of Purkinje cells 1 mm. long and 25 μ thick, this being really a surface 25 μ by 1 mm. in the cerebellum as weighed.

For comparable quantitative results this correction is necessary because it is evident that when the cerebellum shrinks, the line of Purkinje cells shrinks also, and it is necessary to correct both for the change in the length of the line and for the change in the thickness of the section on the slide—it being evident that the 25μ thickness of the section represents a greater thickness in the cerebellum as weighed. The sum of the length and breadth of the section have been used rather than one dimension alone, because careful measurements show that the section is compressed in breadth to some extent when cut on the microtome. The sum of the two measurements consequently gives a more accurate indication of shrinkage.

The number of cells found in a line 1 mm. long and in a section 25μ thick would afford a satisfactory unit for comparing different areas of the same brain; it would not do, however, as a unit for comparing different brains. If the molecular layer of the cerebellum should be removed, leaving the cell bodies of the Purkinje cells intact, it is evident that these would extend in a much convoluted sheet over the surface of the remaining part of the cerebellum. The area occupied by the Purkinje cells in a large brain is thus larger than that occupied by them in a small brain. The sur-

faces of similar solids vary, we know, as the squares of the cube roots of their volumes. A priori we should perhaps not be safe in assuming that large and small cerebella are exactly similar solids, it being possible that small cerebella might be more convoluted than large ones so that the disparity in surfaces might to some extent at least be removed.

Careful examination, however, of large and small cerebella—where the latter are from cases at least one month old—does not justify this a priori assumption. Individual differences do exist, but size has not been found to alter materially the convolution pattern after birth (Berliner, '05). Furthermore, our results based on the assumption that large and small cerebella are similar solids will be found to agree with theoretical expectations.

In order, then, to compare areas which represent the same fraction of the total number of Purkinje cells in different cerebella, it is necessary to take areas which vary as the squares of the cube roots of their volumes. As a convenient indication of volume we have used the weight in grams of the fixed cerebellum, this having been corrected, if necessary, for change in specific gravity. A constant fractional part of each cerebellum, an *equivalent unit area* (EUA), has been secured by taking a line of Purkinje cells as many millimeters long as the numerical value obtained, by taking the square of the cube root of the cerebellar weight in grams—this being from a section cut 25μ thick and corrected for shrinkage as already explained.

If, then, we let

N = the number of cells per EUA
L = the length of the line of cells as projected in millimeters
M = the magnification
C = the total number of cells counted
S_1 = the sum in mm. of the length and breadth of the block as first measured
S_2 = the sum in mm. of the length and breadth of the section on the slide
W = the weight of the cerebellum in grams after fixation then

$$N = \left(C \div \frac{L}{M}\right) \times \left(\frac{S_2}{S_1}\right)^2 \times W^{\frac{2}{3}}$$

or

$$N = \frac{CM}{L} \times \left(\frac{S_2}{S_1}\right)^2 \times W^{\frac{2}{3}}$$

As an example we may substitute the values for Case 15035, Area 3, left hemisphere (table 1).

$$\begin{aligned} N &= \frac{479 \times 30}{2220} \times \left(\frac{82.6}{89.1}\right)^2 \times 126^{\frac{2}{3}} \\ &= 6.47 \times 0.86 \times 25 \\ &= 138 \end{aligned}$$

RESULTS OBTAINED FROM COUNTS

Neurohistologists who have made a study of the Purkinje cells are familiar with the fact that these cells are not evenly distributed, but tend to be more or less irregularly spaced, and this seems to be particularly true for the human cerebellum. In the bottom of sulci they are not normally so numerous as they are at the summits of the folia. It would not be far wrong, in fact, to say that they increase in frequency as one passes from the bottom of the sulcus to the summit of the folium. This is probably to be interpreted as incident to the phenomena of growth. The bottom of a sulcus represents an arrest in growth; the summit of the folium is the last to fill out and develop. It is, then, not surprising that a greater number of Purkinje cells should appear in the region characterized by the greatest amount of growth change.

Considerable variation will be found in the frequency of Purkinje cells, even in adjacent folia. This is especially true of defective brains, less so of normal ones. This makes it necessary to count several hundred cells in order to get a satisfactory average for any given area. Each value given in the tables in this paper is based on an average of about 400 cells, and it is believed that by taking a number so large the effects of accidental selection have very largely been eliminated.

If perfectly satisfactory normal cerebella had been used as the basis for the norms given in table 1, it seems certain that

all the values for the different areas would be somewhat higher than those given there. It has seemed wiser, however, to use the values actually observed rather than to attempt the more or less dangerous expedient of guessing at the extent to which these have fallen below the norm.

The number of Purkinje cells in equivalent unit areas (EUA) of the two hemispheres of the four normal cerebella is shown in table 1.

From table 1 it appears that the cells are closer together in the lobus anterior (area 1) and that they become progressively less numerous in the anteroposterior direction, with the exception that in the lobus biventer, area 6, the number is slightly greater than in area 5.

In the lobus biventer the cells are usually more numerous in the posterior part. This is not shown in the table because the two parts are considered together; the fact is of importance, however, in relation to some of the changes found in subnormal and senescent cerebella, for in our cases the anterior part of this lobe has suffered the greatest amount of degeneration.

TABLE 1

Number of Purkinje cells per EUA in normal cerebella. Areas as in fig. 1. R=right hemisphere, L=left hemisphere

W. I. NO.	RACE	HEMI-SPHERE	AREA 1	AREA 2	AREA 3	AREA 4	AREA 5	AREA 6	TOTAL
14455	W.	R	169	115	147	155	121	114	1566
		L	150	106	125	109	133	122	
15035	B.	R	169	139	151	138	121	127	1655
		L	151	138	138	140	120	123	
15073	B.	R	164	138	118	109	116	145	1608
		L	150	132	121	130	146	139	
14485	B.	R	165	156	118	117	117	112	1697
		L	181	158	160	160	121	132	
Average.....			162±8	135±2	135±10	132±14	125±6	127±5	

Note. The numeral preceded by ± equals one-half the average difference between the right and the left hemispheres.

Differences between right and left hemispheres. A glance at the table shows that in most cases there is a considerable difference between the values for the right and left hemispheres. In three cases the values for the right hemispheres are larger, while in one case the value for the left is the larger. Unfortunately, it is not known whether these cases were right or left handed, and it is consequently impossible to show here a positive correlation between a greater number of cells and superior unilateral skill. As a suggestion, however, it is not improbable that we have here three right-handed individuals and one left-handed. In any case it may be noted that the differences between the right and left hemispheres are greatest in areas 3 and 4, the supposed arm and hand areas, and that they are least in area 2, the neck area. On purely a priori grounds this is what is to be expected if there is a correlation between number of cells and fineness of muscular coördination. The greatest difference between the right and left hemispheres should be in the hand and arm areas, because it is in the two hands that there is normally the greatest difference in motor skill. In the neck area there is obviously less reason for expecting one side to be more developed than the other. However, further studies must be made here on cerebella from cases with known histories before this question can be answered with any assurance of correctness.

SUBNORMAL CASES

In order to compare the relative frequency of Purkinje cells in the cerebella of subnormal individuals, the histories of which have been given on page 232f, with normal ones, counts have been made on areas 1, 3, 4, and 6 of the right hemisphere and on areas 3 and 4 of the left hemisphere. These results are presented in table 2.

It is obvious at a glance that in this group of mental defectives the number of Purkinje cells is notably deficient. The constancy of the results and the wide difference between the values for the normal and those for the defective cerebella as shown by the fact that the latter are only 63 to 78 per cent of the former make it impossible to assume that accident, chance, or

TABLE 2

Number of Purkinje cells per EUA in subnormal cases. Designations as in table 1

W. I. NO.	HEMI-SPHERE	AREA 1	AREA 3	AREA 4	AREA 6	TOTALS
14880	R	99	98	93	106	598
	L		102	100		
15144	R	88	126	122	100	652
	L		114	102		
15145	R	123	85	85	137	630
	L		95	105		
15250	R	105	103	109	127	660
	L		108	108		
15297	R	135	93	89	95	597
	L		88	97		
15299	R	109	92	79	47	503
	L		95	81		
15310	R	85	100	94	98	527
	L		77	73		
15320	R	93	80	95	67	495
	L		85	75		
Average		105	96	94	97	
Per cent of normal as in table 1		63	71	71	78	

personal equation have played any considerable part in determining the differences observed. Moreover, the number of cases is large enough to make it practically certain that typically a decidedly smaller number of Purkinje cells is present in the low grades of feeble-minded individuals. Here, then, we have an anatomical deficiency as a basis of the observed deficiency in motor coördination. To this should be added the fact that many of the cells counted were atrophied and showed consolidation and degeneration of the nucleus to such an extent that they could hardly have been of any functional value. Many

other cells, although not so far degenerated, showed clearly the early stages of the process. With many of these very important elements of the neuromuscular mechanism lacking, and with many more in a degenerating condition, it is inevitable that poor coördination should result.

After the cell counts for the different areas were completed, the clinical histories of the subnormal cases were consulted and an effort was made to see how far the variations in the different areas would give evidence either for or against the localization theory of Bolk. Too much must not be expected from such a comparison, because the case histories were not made with a view to their being used in this manner and they are consequently incomplete. It will be interesting, however, to consider briefly the facts in each case.

No. 14880 shows as compared to the normal the lowest number of cells per EUA in the head area. The personal history shows that he had a small head and poor speech. It is consequently not improbable that the entire head musculature was under-developed and poorly coördinated. The record says he was right handed. The cell count shows a slightly greater number of cells in the left hemisphere. It will be shown later in discussing senescence and paresis that in right-handed individuals the right hemisphere suffers more loss than the left. The case history indicated postnatal degeneration, and this, if true, would account for the smaller number of cells in the right hemisphere.

No. 15144. The history shows a bullet-shaped head and very poor speech. He was able, though, to thread needles and tie his shoes, which shows that his hands at least were capable of difficult motor coödinations. The cell counts show the head area very low, but the arm and hand area nearly normal. This agrees well with the localization theory.

No. 15145. The history shows epilepsy—a degenerating disease—an abscess on the right hip, and a failure in the grip test. The cell counts show the arm and hand areas lower than the others, with the right side lower than the left, this being due probably to degeneration.

No. 15250. The record shows poor speech, atrophy of the jaw, and eye fatigue. The cell counts show the head area the lowest. The grip test shows the left hand slightly stronger and the cell count for area 3 agrees with this.

No. 15297. The record shows a mental age of eight, which, as far as the cerebellum is concerned, would indicate a higher development of the head area with respect to eye movement, speech, and facial expression. The cell count for area 1 is the highest of the eight cases listed. This agrees with theoretical expectations. The record for grip

shows the right hand stronger; the cell count for area 3 agrees with this, but for area 4 is opposite to it.

No. 15299. This is a low-grade case, unable to talk or work; also he was knock-kneed. The cell counts for both hemispheres are very low, and this is especially true in area 6, the leg area (?). A large part of the anterior part of the lobus biventer in the right hemisphere was completely atrophied. The grip test shows both hands about equally weak and there is little difference between the cell counts for the two hemispheres.

No. 15310. The record shows that he was left handed and that a Wassermann test was positive. This probably accounts for the extreme loss of cells in the left hemisphere through degeneration. The grip tests shows the right hand stronger and the cell count shows more cells in the right hemisphere.

No. 15320. The low cell counts here agree well with the low mentality and the general lack of motor power and coördination. The tumor in area 6 of the right hemisphere probably accounts for the loss of ability to walk well after the age of eighteen. Also it is important to note that many of the remaining Purkinje cells were in the process of degenerating. General motor deficiency was consequently inevitable.

In view of the rather meagre clinical data available, it was not expected that a very high degree of correlation would be found between the reported defects in motor coördination and the numerical deficiency in Purkinje cells. Furthermore, a statement of the relative number of cells, if taken alone, is not necessarily a complete indication of functional efficiency; for the cells, although present, may be so far degenerated that they are without functional value. It is consequently interesting to find that in the cases considered there is a considerable difference, even in cell number, between the normal and the subnormal and that the losses in cells agree very well with Bolk's localization theory.

The question naturally arises as to whether this deficiency in cells is due to agenesis or to some toxin or other agency present during intra-uterine life, or to postnatal disease or injury, acting on the cells already formed.

To throw some light on this problem, counts were made on area 3, the arm area, of both hemispheres of six low grade infants. These may safely be said to be of subnormal ancestry; also they can hardly be said to have had the most favorable nutritional conditions during fetal life. How the number of cells in these cases compares with the normal is shown in table 3.

TABLE 3

Number of Purkinje cells per EUA in subnormal infants. Designations as in table 1

W. I. NO.	RACE	AGE	AREA 3	
			R	L
		months		
14428	B.	1	120: 105	
E22	W.	1.5	126: 115	
14250	W.	3.5	106: 120	
E23	B.	5	94: 103	
14215	B.	9	110: 102	
14427	B.	36	107: 111	
Average			110	
Normal			135	
Per cent of normal as in table 1			82	

The sections do not show that cells have degenerated and dropped out, and yet in every case the number of cells is distinctly below normal. The cause of the deficiency must then have acted antenatum. Whether this is to be charged to agenesis or to early destruction is, however, not easily determined. The writer's opinion, based on the evidence at hand, is that the cell deficiency is due primarily to agenesis. Beyond this it is not possible to determine from the present study the etiology of the conditions observed.

In some cases, however, mental and motor deficiency appears to be due to causes which operate postnatum. This is shown in case no. 15214. The cell counts as compared with the normal are shown in table 4.

These values in table 4, the reader will note, are almost normal. Yet the case is a distinctly subnormal one. The clinical history shows that the ancestry is free from feeble-mindedness as far as known; also it shows that the child was normal until nine months of age. At the time he began to have spasms and to show evidences of subnormality, due perhaps to the delayed effects of congenital lues. The cerebellum is of normal weight, but the

TABLE 4

Number of Purkinje cells per EUA in case No. 15214, an example of postnatal arrest of development. Designations as in table 1

W. I. NO.	HEMISPHERE	AREA 1	AREA 3	AREA 4	AREA 6
15214	R	159	124	106	143
	L		147	151	
Average		159	135±12	128±23	143
Normal		162±8	135±10	132±14	127±5

cerebral hemispheres are much below weight, which further indicates that the cause of the arrest took effect after birth.

An examination of the cells reveals on the anatomical side at least a reason for the defect. Many cells are atrophied and are so far degenerated that they cannot have any functional value. Other cells are less degenerated and some are apparently normal, but others have completely disintegrated and have been absorbed. This is clearly a case of postnatal degeneration, but even so, it represents the exception rather than the rule among subnormal individuals. The entire case history shows this.

In this as in the other cases, then, we find that in low-grade mental defectives there is a distinct deficiency, either numerically or cytologically, in a large percentage of the Purkinje cells.

SENESCENCE

It is a familiar fact that very old people usually suffer a considerable loss of motor control. The results of counts made on five cases of senescence of different ages are given in table 5 and show how the cells drop out with increasing age.

The results of table 5 are shown graphically in chart 1. The value for the normal is based on table 1 and the starting-point for the drop in the curve is placed somewhat arbitrarily at the age of forty years.

In connection with this curve it is interesting to note that it is based on the cerebella of two men of superior mentality, on one negro male autopsied in a general hospital, and on one white male and one white female dying at extreme old age in hospitals

TABLE 5

Number of Purkinje cells per EUA in senescent cerebella. Designations as in table 1

W. I. NG.	RACE	SEX	AGE	HEMI-SPHERE	AREA 1	AREA 3	AREA 4	AREA 6	Total
Normal			42?		162±8	135±10 (135)	132±14 (132)	127±5	823
14464	B.	M.	62	R	141	110	98	97	667
				L		111	110		
16107	W.	M.	65	R	104	102	87	100	591
				L		111	87		
14340	W.	M.	79	R	87	80	84	87	500
				L		84	78		
E27	W.	F.	94	R	68	87	78	52	462
				L		88	89		
14544	W.	M.	100	R	58	69	71	29	403
				L		89	87		

for the insane. The uniformity of the curve is consequently surprising. Much speculation might be based on such results as these, but here it will suffice to call attention to two points: first, the average loss in area 1, the head area, as compared with the values from table 1, is relatively the greatest; second, in areas 3 and 4, the right hemisphere suffers more than the left.

PARESIS

It was not originally intended to include any cases of paresis in this study, but as the cerebellum of one case was prepared before the cause of death was known, and as it is of interest because of its similarity in cell losses to the other types of cases presented, it seemed worth while to include it for purposes of comparison. The results of the cell counts for this case are presented in table 6.

Here again we find the head area very low and the right hemisphere much lower than the left. This was a right-handed man and the cell losses have been greatest on the right side.

The figures for cell losses do not of course fully indicate the loss in functional efficiency of such cerebella. Many of the cells that remain are in the process of disintegration and therefore many of those included in the cell count probably have little or no capacity for functioning.

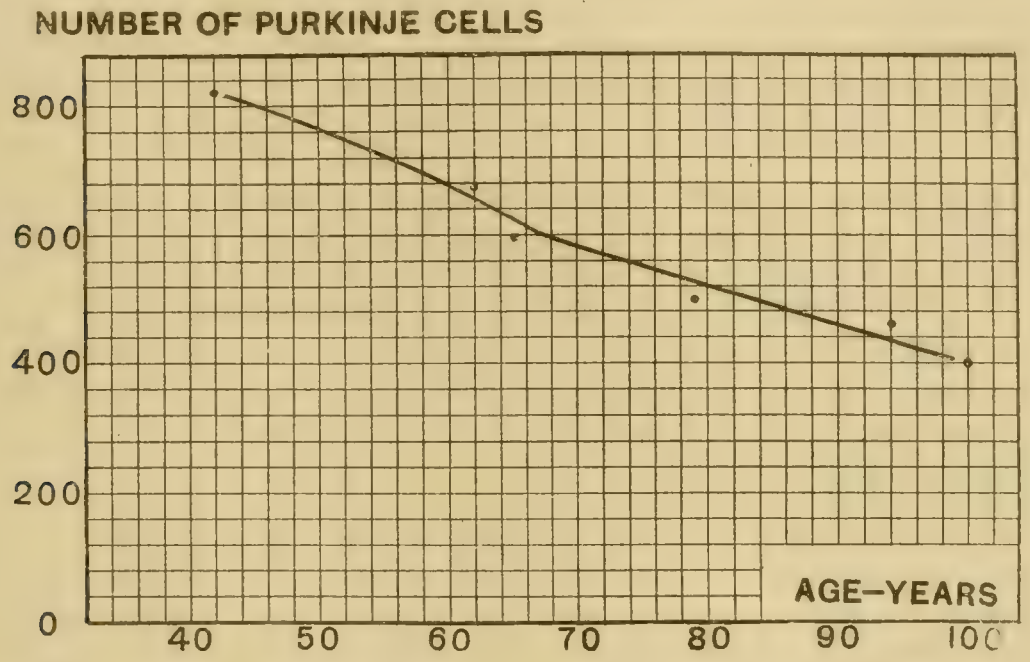


Chart 1 Showing the decrease in the number of Purkinje cells with advancing age. Based on the totals of the four areas given in table 5. The dots show the relation of the six observed values to the graph as drawn.

THE VERMIS

Sections of the vermis were prepared in all cases and a few preliminary counts were made to determine whether or not it would be profitable to make counts on all cases. The results for the tuber vermis in five cerebella—two normal, one senescent, and two subnormal—are given in table 7, and for comparison, the averages of area 4 of both hemispheres are given also.

No significant differences in the relations found in the two sections appear from this table. The sections of the vermis from the other cases were examined under the microscope without counting, and as it seemed clear that they would not differ materially from what had been found in the hemispheres, no further counts were made.

TABLE 6

Number of Purkinje cells per EUA in a case of paresis. Designations as in table 1

W. I. NO.	AGE		AREA 1	AREA 3	AREA 4	AREA 6
14512	33	R	107	99	68	90
		L		133	118	
Normal			162±8	135±10	132±14	127±5

COMPARISON WITH THE CEREBRAL CORTEX

The writer has had occasion to make cell counts on a number of areas in the cerebral cortex, and has found, in agreement with most others who have conducted investigations along this line, that there is ordinarily a distinct deficiency in cells in cases of amentia. Among those authors who may be mentioned here are, for example, Hammerberg ('95), Roncoroni ('05), Tredgold ('03), and Bolton ('03).

The motor area of the cortex in several cases of paresis and also of senescence has been carefully examined, and it is interesting to note that in all of these there has been found a deficiency in Betz cells. How general this particular loss is, the writer does not know; it is, however, important to recognize that disintegration takes place in the cerebrum in a manner similar to that found in the cerebellum. It is therefore not probable that the motor deficiencies observed in the cases studied have been due solely to deficiencies in the cerebellum. Probably in the majority of cases a defective cerebellum is accompanied by a defective cerebrum, and vice versa.

It would be beyond the scope of the present paper to attempt a more detailed statement of the character of the cell changes in these atypical brains; enough has been done, however, if we have succeeded in establishing the numerical differences found in these different types of cases.

TABLE 7

Number of Purkinje cells per EUA in the tuber vermis, compared with the average for area 4

	NORMAL		SENESCENT	SUBNORMAL	
	W. I. No. 14485	W. I. No. 15073	W. I. No. 16107	W. I. No. 15310	W. I. No. 15320
Tuber vermis	157	137	112	83	91
Area 4	138	119	87	84	85

SUMMARY AND CONCLUSION

The main purpose of this paper was to show the numerical differences in Purkinje cells in normal, subnormal, and senescent cerebella.

From the data submitted it is evident that in cases of extreme mental defect due to agenesis or to the early action of toxins during intra-uterine life, there is an evident deficiency in the number of these cells.

Similar reductions in the number of cells due to various causes are found in senescence (and paresis).

In the subnormal cerebella the evidence indicates that the normal number of cells has never been present in a developed form. In the senescent (and paretic) cases, however, the small number is due to disintegration.

The anterior lobe of the cerebellum shows the greatest deficiency in cells in both the subnormal and senescent cerebella.

The biventral lobe shows the greatest variation in both types of cases. In some cerebella it shows the greatest loss of cells and in others the least loss.

The differences between the two hemispheres in respect of cell number average less in subnormal cerebella than in normal ones. This probably has a relation to the differences in the degree of unilateral dexterity found in normal and subnormal individuals, i.e., normal people are usually more distinctly right (or left) handed than are the subnormal, who tend to be more ambidexterous.

The deficiency in cell number affords in large measure an explanation of the motor defects found in subnormal individuals. It shows, furthermore, that in idiocy and in imbecility we may expect to find the whole brain defective rather than the frontal lobes only, while the higher grade of defectives (morons) probably show very slight deviations from the normal.

Further studies in this field on better material with better-known clinical histories in which is included a study of blood-vessels, nerve fibers, neuroglia, and a cytological study of all the important types of cells are necessary to bring out the more detailed differences between these types of cerebella.

For a review of the literature on the clinico-pathological study of the cerebellum with a detailed report of a single case see Archambault ('18).

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, MARCH 17

THE EFFECT OF OVER-ACTIVITY ON THE MORPHOLOGICAL STRUCTURE OF THE SYNAPSE

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FOURTEEN FIGURES

INTRODUCTION

Investigations on the histological manifestations of the nerve cell in fatigue have long been familiar to us. Numerous authors have brought contributions to this important and interesting subject. If we take for granted, as Sjoeval declared, that the alteration of nerve cell in tetanus is to be regarded as the effect of activity, the number of references becomes even more abundant. It would suffice to point to those works of Hodge (14, 15, 16), Vas (33), Mann (23), Lugaro (29), Sjoeval (32), Dolley (17, 18, 19), and many others. There is almost a complete agreement on the point, that over-activity causes appreciable histological alterations in the nerve cell. Kocher (19), who studied the same subject in our laboratory, could not, strangely enough, find any qualitative and quantitative changes in the histological characters between the fatigued and resting nerve cells. Recently Saito undertook an exploration in the same line in this clinic, but the results are not published yet.

Compared with the abundant researches on the neurocytological manifestations, there has been no attempt made to investigate the histological alteration of the synapse in fatigue, as far as I know. Not only in regard to over-activity, but also in other pathological conditions, the histopathological changes of the synapse have been the topic of very few investigations. And no wonder, for the structure of the synapse had not yet been conclusively demonstrated even in the normal condition, despite

many investigations by different authors (Golgi, Bethe, Held, Bielschowsky, Auerbach, and many others). The lack of our knowledge concerning the pathological manifestations in the synapse and especially of experimental pathological data seems to be dependent on the difficulty of technique on the one hand and inaccessibility of adequate material on the other. In my recent publication (24), I described more minute structures of the synapse of the Mauthner cell of teleosts (bony fish). Perhaps I have not made the structure of the synapse clear beyond discussion; however, my results have been sufficiently definite for me to attempt experimental work on the pathological condition, especially in fatigue. Careful and thorough investigation on this topic led me to very interesting results, which throw light on the histological condition of the synapse in functional activity, although it was forced far beyond the physiological limit. In the present paper I will describe mainly the manifestations in the synapse as it was not my purpose to investigate the neurocytological alterations of the Mauthner cell itself. But wherever notable manifestations of the cell body appear, they will be described as well as the findings of the synapse. To Dr. Adolf Meyer I beg to express my high appreciation here for his help and kind suggestions in this study.

MATERIAL AND METHODS OF STUDY

In the present investigation Ameiurus nebulosus was the material of experimentation; Carassius auratus, which offered me many interesting results in my previous work, proved to be unfavorable material, owing to the fact that it is weak and often dies before it comes to the utmost exhaustion. It was always attempted in this experiment to work with strict control of the normal structure of the Mauthner cell as well as its synapse, and care was always taken to avoid the formation of artefact as much as possible.

As a resting non-fatigued control, which was provided in each experiment, I used a fish of about the same size which was kept in a resting condition during the experiment of the other fish. The body length of the fish used varied from 5 to 7 inches.

The experiment consisted in forced activity of the fish, carried to the most advanced stage of fatigue; the experimental fish was placed in a jar 7.5 inches in diameter and about 8.5 inches in depth and then the water in the jar was continually stirred by running water, which came with high pressure from a faucet. The fish swims in the stirred water, trying all the time to hold its equilibrium. According to Bartelmez (3), the Mauthner cell participates in the equilibratory reflex; so it was supposed that in this experiment the Mauthner cell would be forced into continuous activity. The duration of the experiment varied remarkably in each fish (from 24 to 98 hours). At first the fish swims actively and is able to hold its equilibrium very well, but gradually it gets tired and in the final stage of the experiment it is deprived of the ability of balancing, so that it is moved passively tumbling around in the stirring water. By this time if we stop the running water for a moment, the fish would lie on its back or on one side, showing no attempt to maintain its upright posture. As the sign of utmost exhaustion, I chose a test, which consisted in holding the fish upside down by its tail in the water as well as in the air. In the most advanced stage of exhaustion the fish did not flap at all even in this test.

The fish was then decapitated and bled, the brain was quickly but carefully dissected out and fixed in 10 per cent formalin, formol-Zenker fluid, and alcohol (95 per cent), respectively. The resting control fish was killed at the same time and the brain was manipulated in quite the same way as the fatigued brain. It must be emphasized also that the material was always fixed fresh and that material from fish which died was not examined.

From the material thus obtained, the following preparations were made with the same technique as was described in my recent publication:

1. Thionin-eosin preparation (1 series each of normal and fatigued brains).
2. Toluidin-blue preparation (5 series each of normal and fatigued brains).
3. Heidenhain preparation (6 series each of normal and fatigued brains).

4. Levaditi preparation (14 series each of normal and fatigued brains).

5. Bielschowsky preparation (6 series each of normal and fatigued brains).

6. Cajal preparation (5 series each of normal and fatigued brains).

Besides these I used the following stains in the present work:

7. Scharlach stain (5 series each of normal and fatigued brain). The fish brain was fixed in 10 per cent formalin twenty-four hours, rinsed with water, cut at 15μ with the freezing microtome. The sections were stained in saturated solution of Scharlach R in 70 per cent alcohol, rinsed with distilled water, counterstained with diluted Ehrlich's hematoxylin solution, washed again, and mounted in glycerin. As there is only one pair of Mauthner cells in a fish brain, it was rather hard to get the sections in which the Mauthner cell is found.

8. Mallory preparation (5 series each of normal and fatigued brain). In this preparation the brain was fixed first in 10 per cent formalin twenty-four hours and then placed in formol-Zenker fluid twenty-four hours. Paraffin sections 5 to 8μ thick were stained with a diluted solution of Mallory's hematoxylin.

9. S.-fuchsin-light green stain (5 series each of normal and fatigued brains). Brains were first fixed in 10 per cent formalin twenty-four hours and then placed for eight days in the chromic acid acetic acid mixture. Paraffin sections of 5μ were treated as follows: 1) Remove paraffin from the sections and pass to 96 per cent alcohol. 2) Place sections for one hour in a saturated aqueous solution of S-fuchsin in the incubator at 58°C. 3) Wash twice with distilled water, till no more stain comes out of the sections. 4) Dip the slides in motion 10 to 20 seconds in the following solution:

Saturated alcoholic solution of picric acid	30
Aqua destillata	50

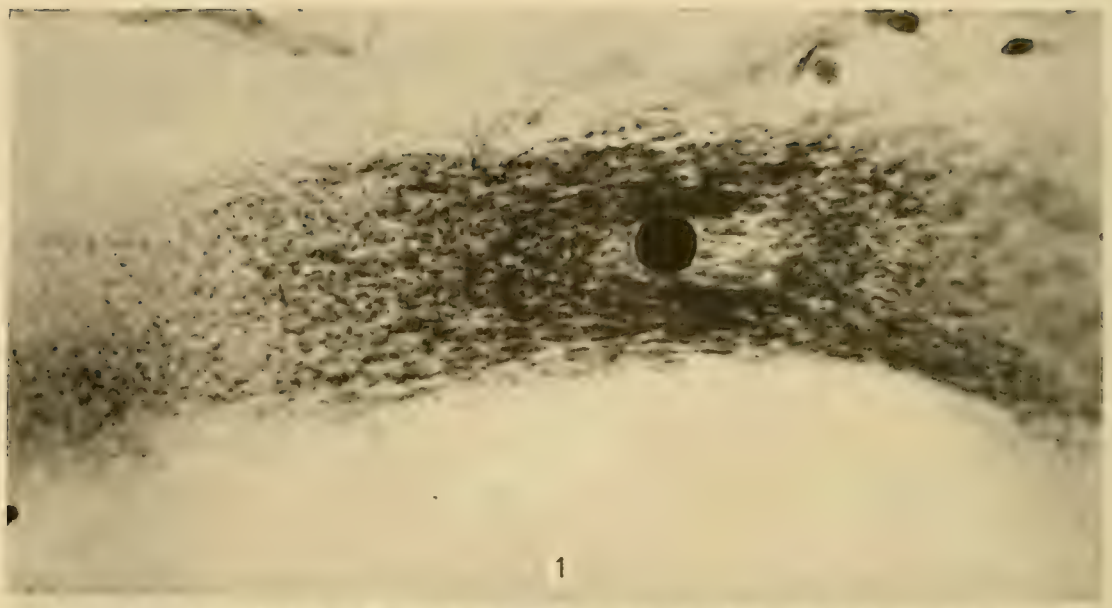
5) Rinse carefully twice in water. 6) Place the slides in a saturated aqueous solution of light green twenty minutes. The sections were then washed with water, dehydrated very quickly and passed into xylol.

In all 122 series of normal and fatigued brains form the basis of the present article.

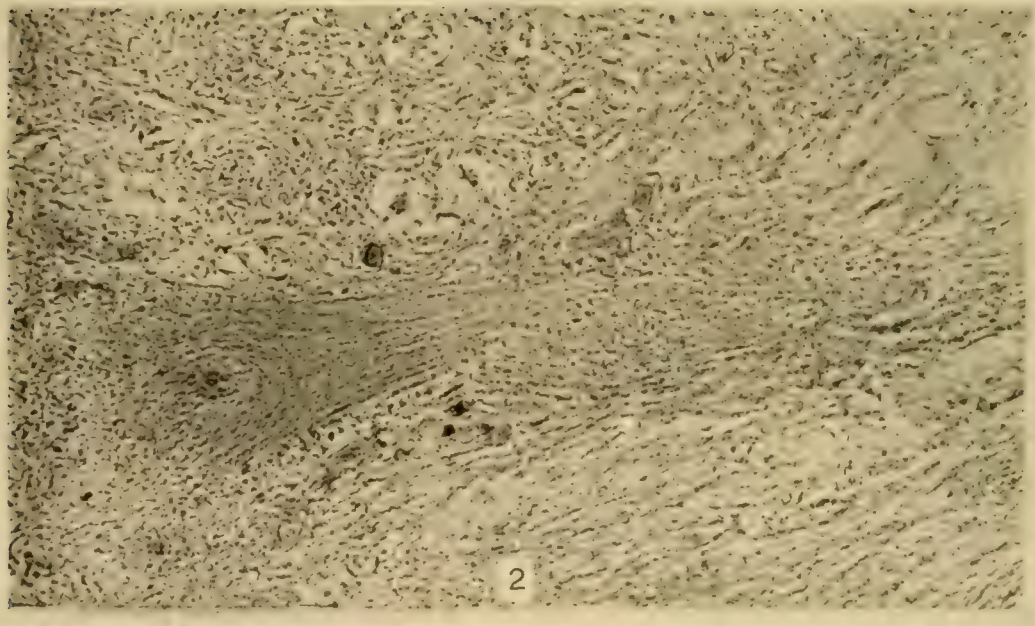
ON THE INTERNAL MORPHOLOGY OF THE MAUTHNER CELL AND ON THE MINUTE STRUCTURE OF THE SYNAPSE

Under this heading I will make a few notes on the internal morphology of the Mauthner cell (figs. 1 and 2) and on the structure of the synapse, which are necessary as the foundation of the following statement and were not yet described in my recent publication. Nissl bodies are distributed evenly through the cell body and bases of the dendrites, leaving free only the axone hillock. They are relatively small as compared with those in the motor cells and very numerous, as Bartelmez (3) stated. The Nissl substance is found in the shape of variably long striae and is arranged generally parallel to the contour of the cell body and in part also to the surface of the nucleus. The remaining stainable substance is irregularly scattered and is more or less short; some of it is spheroidal. The spindles were found especially on the surface of the cell and in the larger dendrites. The so-called nuclear caps did not come to my observation. The axone hillock is entirely free from stainable substance and marked off by a tolerably sharp-curved plane from the granular protoplasm of the cell body and shows at its margin a layer of especially fine granules. The nucleus of the Mauthner cell differs in no essential from the typical nuclear structure of the nerve cell.

As was precisely stated in my recent communication, the synapse of the Mauthner cell is penetrated by the Golgi network, which is formed by the arborization and the reunion of the delicate processes of the neuroglia cells in and about the 'axone cap.' It was also accepted that the Golgi network is to be attributed to that category of the neuroglia tissue, which Held (24) termed as the reticular glia tissue formed by the somewhat modified plasma of the glia cell. Furthermore, I paid special attention in that paper to the histological structure of the nervous elements of that synapse and the relation of the latter to the Golgi net. We may therefore pass directly to the description of the finer structure of the glia cells themselves and the condition of the capillaries in this synapse.



1



2



3

The neuroglia nucleus is surrounded by a variously wide border of protoplasm, which latter sends protoplasmatic processes in different directions. The process itself ramifies and becomes more and more delicate, until it passes into the beams of the Golgi net or becomes attached to the wall of a capillary. This picture could very clearly be observed in the Levaditi preparations, Heidenhain preparations of formalin material, Mallory and acid-fuchsin-light green preparations. In the Heidenhain and thionin-eosin preparations of formol-Zenker material a similar condition was demonstrated, although it was not so clear as in the above-mentioned preparations.

The protoplasm of glia cells was brought out favorably in the thionin-eosin preparations, Heidenhain preparations, Mallory and acid-fuchsin-light green preparations. There are two different types of neuroglia cells; in one type the protoplasm is very scanty, so that the glia cell shows a small round cell body, while in another type we find a large mass of protoplasm around the nucleus. The neuroglia nuclei are sometimes connected with each other not by means of the Golgi-net substance, but by a variously broad mass of granular protoplasm. I should interpret this as the result of amitosis which is observed now and then in the synapse. It must be positively emphasized here, however, that all the glia cells of the synapse belong to one reticulum and that there is no cell individual among them in normal brains. The structure of the glia nucleus hardly calls for a description except that sometimes it shows evidences of amitosis, as Bartelmez (3) also stated. Capillaries are found here and there in and about the synapse of the Mauthner cell. They do not offer anything particular in their

The figures 1 to 8 are the unretouched photomicrographs taken from different preparations of both the control and the fatigued Ameiurus brains. In figures 2 and 5 an apochromatic Zeiss ocular no. 4 and Zeiss objective D were used; others were taken with the same ocular and a Zeiss immersion $\frac{1}{12}$. The length of bellows was 60 cm. in all the photomicrographs. The figures 6 to 14 were drawn from different preparations of fatigued Ameiurus brains, using the Abbe camera lucida. (Zeiss apochromatic ocular no. 4, Zeiss oil-immersion $\frac{1}{12}$, tube length 20 cm.)

Fig. 1 Toluidin-blue preparation (alcohol material) (control fish).

Fig. 2 Thionin-eosin preparation (formol-Zenker material) (control fish).

Fig. 3 Toluidin-blue preparation (alcohol material) (fatigued).

structure and consist of endothelium and adventitia with very few nuclei. As already remarked, the adventitia of the capillary is connected with the bases of the reticular beams; but I was not able to distinguish the perivascular limiting membrane of Held as a membrane separated from the adventitia, so that I could not find the so-called perivascular lymph space between them in normal fish brains.

THE HISTOLOGICAL MANIFESTATIONS OF THE MAUTHNER CELL IN FATIGUE

To recapitulate and discuss the results of other authors here would go beyond the purpose of the present work; my remarks will be restricted to my main results of investigation.

The cell body of fatigued cells was found either in the state of turgescence (figs. 3 and 4) or of shrinkage (fig. 5); in the former case the cell border was convex between the dendrites and the dendrites appeared shorter, whereas in the latter the cell border was concave and the dendrites looked longer. I agree with the opinion of Vas (33), Mann (23), Lugaro (21), Pugnat (26) and Holmgren (17), that the enlargement of the cell body is to be considered as the manifestation of activity and the shrinkage as that of exhaustion. In this way the results of Hodge (14, 15, 16), which deviate from those of others, might well be interpreted. Dolley (7, 9) described the fluctuations of the size of the cell body in the course of activity, but I have a little doubt about his statement that in later stages the absolute size of the cell body increases steadily to the end. The alteration of the Nissl substance manifested itself in more or less advanced stages of chromatolysis, as was described by Vas (33), Lambert (20), Mann (23), Lugaro (21, 22), and many others, and thereby the cytoplasm was stained variously deeply (figs. 3, 5). The Nissl bodies were found in a state of fragmentation, shortening, and irregular distribution; in the advanced stage of chromatolysis they were reduced to fine granules or even to a homogeneous substance. Sometimes I observed the central beginning of the chromatolysis, as was stated by Vas (33), Mann (23), and, in tetanus, by Sjoeval (32).



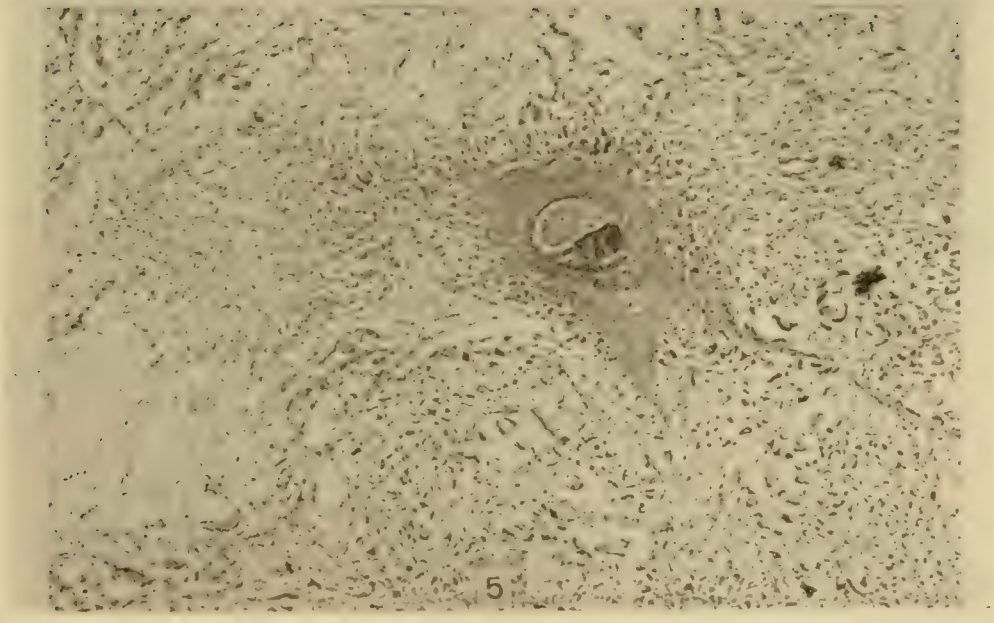


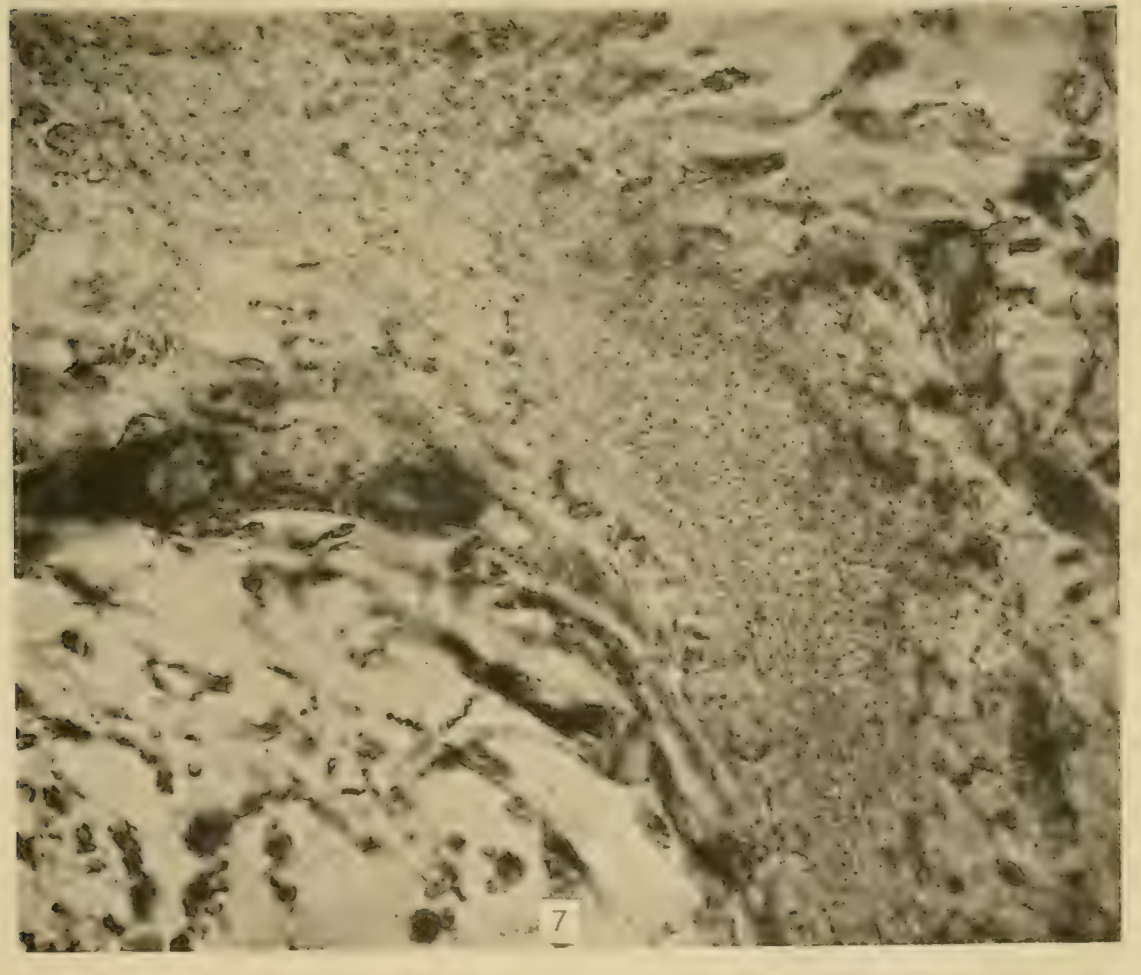
Fig. 4 Toluidin-blue preparation (alcohol material) (fatigued).
Fig. 5 Thionin-eosin preparation (formol-Zenker material) (fatigued).

The nucleus was sometimes located in the center of the cell body, but in many cases it was situated more or less eccentrically, as was described by many others—Vas (33), Lambert (20), Sjoeval (32), Holmgren (17). Morphologically, the nucleus showed now and then no particular change, but in other cases it was found either swollen or shrunken. The swollen nucleus appeared large and round and showed a smooth nuclear membrane, whereas the shrunken nucleus showed irregular shape and a crenated membrane. Vas (33) observed always an enlargement of the nucleus, while Hodge (14, 15, 15, 16) found regularly the shrinkage of the latter. Lugaro (21), Mann (23), Pugnat (26), and Holmgren (17) declared on the basis of their study that activity causes turgescence followed by shrinkage in exhaustion. Sjoeval (32) denied the pathological significance of this finding for the reason that he observed those nuclei also in the cell which showed no change in particular. I think those manifestations of the nucleus are to be attributed to different stages of activity; Dolley (9) also declared that the nucleus shows fluctuations of size in the course of activity.

The nucleolus was found mostly eccentric in the nucleus, although this was the case also in some resting nerve cells. It was sometimes observed swollen (fig. 4); in other cases it showed an irregular shape, oblong or angular (fig. 5). Mann (23), Lugaro (21), Luxemburg (22) observed the enlargement of the nucleolus during activity; it disappears later in exhaustion (Mann (23), Luxemburg (22)). Goldscheider and Flatau (12, 13), and Sjoeval (32) confirmed this in tetanus. Mathes (25) observed the nucleolus of angular shape as I did; the objection, that it might be the deceptive appearance caused by the granules above or beneath the nucleolus, could not come in question in my cases.

As a most peculiar manifestation of the nucleus figure 5 was presented; the nucleus is very large compared with the size of the cell body, the nuclear membrane is marked sharply and the nucleolus itself is small and shows an ellipsoid shape. Besides that there is a deeply blue-stained substance of triangular shape and the remaining space of the nucleus is filled with compact acidophile substance. The nucleus shown in figure 6 might come under





Figs. 6 and 7 Levaditi preparation (fatigued).

the same category of nuclear change; we observe here besides the nucleolus a rod-shaped substance which shows a staining reaction similar to that of the nucleolus, although I am not sure about this, as it is from a Levaditi preparation. As far as I know, no such picture of the nucleus has been described before; figure 8 of Dolley's (7) publication demonstrates a cell, the nucleus of which shows a deeply stained spheroidal substance besides the nucleolus, but Dolley did not mention anything about that in the text or in the description of the plate. Whether this kind of manifestation of the nucleus is to be regarded as the alteration of double nucleolus, which latter is met not infrequently, or can be attributed to a special appearance of the nucleus in fatigue, I cannot tell.

Holmgren (17), Sjoeval (32), and others found the stainable substance massed about the nuclear membrane, and forming either an irregular or a complete ring. I also observed the same phenomenon in many cases (fig. 3); the nuclear membrane was outlined by a delicate blue-stained line or a large mass of stainable substance in a different portion of its circumference, giving the picture of a half-moon. Sjoeval and Holmgren interpreted this as a restitution phenomenon of the tigroid substance; Dolley (7) also regarded it as a sign of greater nuclear activity. I agree with the opinion of these authors. Holmgren (17) observed besides, that the nucleolus and the nuclear granulation emigrate from the nucleus into the cell body. The emigration of the nucleolus never came to my observation; the case, however, from which figure 4 was reproduced may indicate the emigration of stainable substance from the nucleus into the cell body. The nucleus as well as the cell body is swollen in this case, and the nucleolus is also extremely swollen, and we find many blue-stained granules going out of the nucleus into the cell protoplasm. On the other hand, I found also the accumulation of the acidophile substance in the nucleus (fig. 5). On the basis of these findings I should agree also with Holmgren, who came to a conclusion that a mutual interchange of substance takes place between nucleus and cell protoplasm in activity. On the ground of Richard Hertwig's doctrine of nucleus-plasm ratio, Dolley (6, 7, 8, 9) measured the size of cell

body and nucleus of nerve cells in activity and divided the cells into many stages of alteration. As I did not undertake the measurement of the cell body and nucleus, I will not go further into details of Dolley's work; but his method of division is, as he himself admitted, an arbitrary one, and I found many cells, which cannot be assigned to any of his stages. Furthermore, I am afraid that in his interpretation of things, facts are linked with hypothetical considerations which are not directly observable. I will be satisfied in the present work, if I can make sure that the Mauthner cell, the synapse of which is the material of this study, manifests appreciable changes in fatigue.

THE HISTOLOGICAL MANIFESTATIONS OF THE SYNAPSE IN FATIGUE

In these experiments which consist in forced activity, although it goes far beyond the physiological limit, attention was directed from the first, not only to the nervous constituents of the synapse directly, but also and especially to the manifestations in the glia tissue, which shows the changes of the functioning nerve tissue in an indirect way. It was hoped that through the study of the histological manifestations in fatigue some light would be thrown upon the problem, concerning the function of the neuroglia cells in the state of physiological activity. I shall first describe the findings in the synapse of the Mauthner cell in fatigue and later go over to the consideration of the significance of the manifestations.

A. Manifestations in the Pericellular Reticular Structure of the Synapse

The glia reticulum of the axone cap and of the cell surface presented itself in fatigue in a more or less advanced stage of deviation from its normal configuration. In the Levaditi preparations, which bring out the net figure very clearly and sharply in a dark brown or black color, the alteration of the net configuration is most distinctly noticeable. Figures 3 and 4 of my recent publication (24) demonstrate the normal condition of the glia reticulum in the Levaditi preparation. The Golgi network, which is visible on the cell surface (3) as well as in the axone cap (4),

stands out sharply, and the net beams are demonstrated in rigid dark lines.

In many cases of fatigue this net configuration appears more or less irregular and less sharply marked. The net beams present themselves swollen and thick here and there; in other places they are thinner than usual and in some places even broken up. The substance of the net beams looks loose and less compact and in the extreme state of decay the net beams are reduced to variably large amorphous corpuscles and look not unlike silver precipitates distributed irregularly in the synapse.

Figure 7 was reproduced from the Levaditi preparation of a fatigue case; it shows the surface section of a slightly shrunken Mauthner cell and the cell surface is covered partially with the Golgi network. The reticular structure of the synapse stands in this case in a more advanced state of alteration. The spheroidal or star-shaped structures, which correspond to the nervous terminal feet, are demonstrated very distinctly in the nodal points of the Golgi network (especially clear in the lower part of the figure). Now, the net beams connecting these terminal feet with each other are in part marked tolerably sharply, but most of them are swollen and are not clean cut. Some others look, on the contrary, very thin and loose or even broken up. Here and there we find the terminal feet, which lie isolated on the cell surface, as a consequence of the breaking up of the radiating net beams. The reticulum of the axone cap (upper part of the figure) is in a similar state of alteration; the net beams are extremely swollen and loosened and appear thick. The reticular figure is partly well preserved, although we find here and there the decay of the net beams.

Figure 8 was produced from another case prepared by means of Levaditi's method; the Golgi network in the axone cap as well as on the cell surface shows essentially similar changes. The net figure here and there is preserved tolerably well, but the meshes are irregular as compared with those in the resting condition. In other parts of the synapse the net beams show a more or less advanced state of alteration and some beams are really reduced to amorphous black fragments.

The above described findings were repeated in a different intensity in many cases of fatigue by means of Levaditi's method, although there were several cases with negative findings in the synapse. It must be emphasized here that in the resting control animal the pericellular reticular structure was always brought out clearly and sharply. Of the manifestations of the glia cells in the synapse I shall give a more precise description later. As espe-



Fig. 8 Levaditi preparation (fatigued).

cially worthy of note here, I want to discuss the relation between the glia cells and the reticular glia structure. The protoplasm and the protoplasmatic processes of the neuroglia cells increase and swell in mass, as will be mentioned below. Some processes show their relation to the reticulum even in their swollen condition, but some of the processes are no longer connected with the glia reticulum. Some of them fall to pieces so that we find protoplasm masses of different shape and size around the glia cells.

Some glia cells even lie freely in and about the synapse; to this I shall come back again.

In the thionin-eosin preparations of the formol-Zenker material it is very difficult to find such delicate alterations of the reticular structure. In a slight alteration it is almost impossible to distinguish the difference between the resting condition and the fatigue case, so far as the net figure is concerned. In the most advanced stage of alteration, however we are able to find similar manifestations as those described in Levaditi preparations, although it is not so easy to see as in these preparations. Figures 2 and 5 were reproduced from thionin-eosin preparations. In figure 2, which demonstrates the resting condition, we find regularly arranged dark points around the cell, which evidently represent the net beams of the pericellular reticulum. In the fatigued cell (fig. 5) we find around the cell body a number of similar dark points, which are, however, scattered without any order at the cell periphery. The microscopic observation revealed clearly a change of pericellular network similar to that described in Levaditi preparations; the net beams were in part swollen and others broken up. The findings of the glia cells I shall describe later. In the Heidenhain preparations and other preparations a similar manifestation of the reticular structure was observable, although it is not so clearly demonstrable as in the Levaditi preparations so that we can find the alteration only in a far advanced state.

B. Manifestations of the neuroglia cells in the synapse

Among the neuroglia cells of the synapse of the Mauthner cell I found in many cases Alzheimer's amoeboid glia cells (figs. 9, 10, 11, 12, and 14). These amoeboid glia cells, as characterized by Alzheimer (1), show a large protoplasmatic cell body with a special morphological structure and small dark nuclei, and in typical

Figs. 9, 10, and 14 Thionin-eosin preparation (fatigued).
Fig. 11 Acid-fuchsin-light green preparation (fatigued).
Fig. 12 Mallory preparation (fatigued).
Fig. 13 Scharlach R stain (fatigued).



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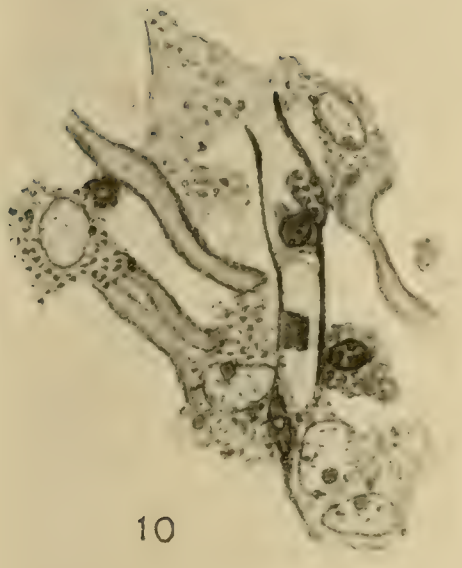




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forms they have striking resemblance to an amoeba. Besides the typical forms I found many others which do not resemble amoeba.

Before I pass to the description of the amoeboid glia cells, I will pay some attention to the manifestations of glia cells which go hand in hand with the appearance of the former. Although not so numerous, we find glia cells in every stage of regressive and progressive change (figs. 9, 10, and 14). The regressive nuclei appear sometimes extremely swollen and pale and at other times they show a zigzag shape and deep stain. The homogeneous stain of the nucleus and protoplasm, the breaking up of the nucleus into spherules or small masses are other histological properties of the regressive nuclei. Furthermore, I observed in the same sections production of young amoeboid glia cell; karyokinesis of the glia nucleus was found now and then, and amitosis of the nucleus, observable occasionally in the physiological condition, seems to appear oftener in fatigue preparations (fig. 6).

In young amoeboid glia cells the shape of the cell body is simple and we find sharply marked protoplasm around the nucleus. In older cells, however, the protoplasm grows larger and sends processes of irregular shape in different directions. The process itself has at first a simple shape, but later it shows a more or less complicated shape; sometimes I observed that the glia cells send the processes to nerve fibers or capillaries, holding the latter between their ramifications. Generally speaking, the amoeboid glia cells were found relatively more numerous near the blood-vessels than in the other parts of the synapse. In young amoeboid glia cells the protoplasm is first quite homogeneous, but in the further course of life many kinds of manifestation become noticeable in the cell body.

In the Mallory preparations (fig. 12) the protoplasm of large amoeboid glia cells contains variously large vacuoles; the size of the vacuoles varies considerably, but generally speaking they are not very large in my preparations. The number of the vacuoles is also variable with the size and age of the cell. The content of the vacuoles is quite clear in my preparations; I assume that these vacuoles are lipoid cysts, the content of which was extracted in the process of embedding. In my thionin-eosin preparations

(fig. 10) and fuchsin-light green preparations I could also find those vacuoles. Besides these I observed in the Mallory preparations dark violet or blue-stained granules in the cell protoplasm. There is no doubt that these granules are identical with the methyl-blue granules of Alzheimer (1). This kind of granules varies considerably in size, but in any one cell they are in general of similar size as Alzheimer described. With the production of this kind of granule the loosening of the protoplasm structure of the amoeboid glia cells takes place. In the acid-fuchsin-light green preparations, in which the methyl-blue granules cannot be brought out, the cell body shows a granular or bubble-like appearance in this stage. At the same time marked changes become noticeable in some nuclei; they are stained either homogeneously deeply or remarkably pale. Even the neuroglia cells in the synapse, which are not in possession of the proper attributes of an amoeboid glia cell, but have long narrow processes instead of a large protoplasm mass, sometimes display alterations; then the processes look as though they were dissolved into granules, which show the same staining reaction as the methyl-blue granules.

In the acid-fuchsin-light green preparations (fig. 11) the amoeboid glia cells were brought out very clearly; in the evenly green-stained cell protoplasm the Alzheimer fuchsinophile granules appeared as large red spherules. The number of these granules varied in different cells; some large and old cells have granules scattered through the whole protoplasm. The sizes of the granules are almost equal and the considerable size distinguishes these granules from the fine fuchsinophile granules, which are only occasionally observable in the normal glia cells. As already remarked, I found also in this preparation vacuoles of different size in the cell body of the amoeboid glia cells. The contents of these vacuoles in my preparations were always clear; the large lipoid cysts with yellow substance described by Alzheimer (1) did not come to my observation. The Alzheimer light green granules were not observed either in my preparations.

In my thionin-eosin preparations (fig. 10) of formol-Zenker material I found also a number of typical as well as atypical amoeboid

glia cells, which were met more frequently around the blood-vessels than in the other parts of the synapse. Large and old cells showed vacuoles of different size in their bodies. Some of these glia cells showed another kind of granules, which were demonstrated by means of the thionin-eosin stain in a characteristic metachromatic or more or less blue-violet color. The granules fill the cell body as well as the processes; the size is variable, but in any one cell they are of almost equal size. The shape of these granules is round or that of irregular lumps. Another characteristic of this kind of granules is that in the illumination by electric light they are especially beautifully observable. In the space around the blood-vessels and also in other parts of the synapse I often noticed a group of these granules; this is to be interpreted as the section of a cell or its process, bearing this kind of granules. As far as my observation went, these granules do not lie freely in the tissue.

What is the nature of these granules? Reich (27, 28, 29, 30) demonstrated in the Schwann cells of the peripheral nerve fibers rod- or comma-shaped fairly large granules (π-granules), which were brought out in a characteristic metachromatic stain by means of thionin, toluidin-blue or kresyl-violet, and he identified these granules with the protagon of Liebreich on account of the similarity of the staining reaction and of the solubility in warm alcohol (45°) and in warm ether. He found, moreover, that they are soluble also in warm xylol, that they are not at all stainable in acid stains, and also that they are especially beautifully observable in the illumination by electric light.

Later, in certain pathological conditions, Alzheimer (1) demonstrated in the neurogolia cells granules which gave a characteristic metachromatic basophile stain by means of toluidin-blue and thionin; he identified these granules with the π-granules of Reich and called them metachromatic basophile granules, although the granules differed somewhat from the π-granules morphologically. I did not test the properties of solubility of the granules, which I observed in the glia cells; but on the basis of their staining reaction and morphological characteristics I assume that they are identical with the metachromatic basophile

granules of Alzheimer. Whether these granules consist of protagon or not, is another question; recent studies raised doubt against the real existence of protagon as a uniform substance. (Rosenheim, Tebb, Thudicum, cited in (18)). I should add here that I always used bergamot oil instead of xylol in the process of paraffin embedding.

Alzheimer (1) declared that he did not find, or he found at the most only indications of, these granules in the amoeboid glia cells; but as far as my observation went, I found a number of amoeboid glia cells with this kind of granules. The finding that these granules are observable in the cells of blood-vessels, as will be described later, and in the glia cells around the blood-vessels relatively more numerously, and the fact that in Scharlach stain fat drops are found in those cells, as will be related below, make one assume that they are transported toward the blood-vessels and that these granules give rise to the production of fat as Alzheimer did. Reich assumed that the appearance of this kind of granules has a relation to the decay of the myelin sheath; according to Alzheimer (1), it is not, however, necessary for the appearance of this kind of granules. As the neuroglia cells of the synapse of Mauthner's cell lie mostly at the border of the axone cap, where the nerve fibers lose their myelin sheaths, I cannot decide this question from my own observation. Moreover, as the granules appear only in fatigue, it is probable that they have something to do with a pathological nutrition condition of the nerve tissue; the fact that they are a catabolism product is acceptable because they are transported toward the blood-vessel, and it is also probable that they are changed into fat, just like the other catabolism products.

On the basis of the above-described facts, it is quite clear that in fatigue a number of amoeboid glia cells are produced in and about the synapse, which carry different kinds of catabolism products in their cell body as well as in their processes. As already repeated, I found these amoeboid glia cells relatively more numerous around the capillaries in and about the synapse. It was also remarked that many a conglomeration of metachromatic basophile granules was demonstrated around the blood-vessels

with a different size and shape (fig. 10). This was also the case with the methyl-blue granules and the fuchsinophile granules. All these granules were embedded in the evenly and lightly stained ground substance, which is to be interpreted as the section of the cell body or the process of the glia cell. As far as my observation reached, these granules did not lie free in the space around the blood-vessel or in the tissue.

The cells of the adventitia of blood-vessels in the normal brain show very little protoplasm around their nuclei; but in fatigue cases, when a number of amoeboid glia cells are present around the vessels, they show a larger mass of protoplasm. In the thionin-eosin preparation I observed occasionally the metachromatic basophileg ranules in them. Scharlach R (fig.13) revealed many fat drops in the latter. It must be added here that in the neuroglia cells around the vessel and in the synapse fat drops were demonstrated within the protoplasm. According to my observation, there was, however, very little fat lying in the tissue or in the space around the vessel.

THE 'FUELLKOERPERCHEN' OF ALZHEIMER

After the description of the changes of the glious reticulum and the glia cells of the synapse, one more manifestation, which goes hand in hand with the appearance of the amoeboid glia cells is to be mentioned. I have already described that in sections in which a number of typical amoeboid glia cells were observable, many a protoplasm mass of different size and shape appears in the perivascular space and in the other parts of the synapse. These protoplasm masses were found to be sometimes homogeneous and sometimes granular and they occurred usually near the amoeboid glia cells. Some of these masses must be regarded as the section of the cell body or the process of an amoeboid glia cell (fig.10); but some of them show evidently that they were cast off from the body of the amoeboid glia cells (fig. 15). It was also related above that the processes of the glia cells, which are not in possession of the proper attributes of an amoeboid glia cell are broken up in their substance and are reduced to fragments. I assume that I

can homologize these protoplasm pieces with the 'Fuellkoerperchen' of Alzheimer (1); as far as the origin of these corpusceles is concerned, I agree with Alzheimer, in so far as the reticular beams of glia tissue swell and are loosened in their substance so that finally they fall here and there to pieces. The probability of a postmortem alteration cannot come into consideration; it must be emphasized here that the fish brains were always fixed in their fresh condition.

THE RELATION OF THE AMOEBOID GLIA CELLS TO THE GLIA RETICULUM

As stated before, the glia reticulum of the synapse of the Mauthner cell in fatigue was found in a more or less advanced state of deviation from its physiological configuration. The net figure appeared less sharp and the meshes were found more irregular; the net beams were observed either swollen or lessened in thickness. The substance of the net beams appeared loosened, and in the state of extreme deterioration the net beams were here and there broken up and reduced to fragments so that in some parts of the synapse the net figure was no longer in evidence. The question now arises whether these manifestations of the pericellular reticulum are to be attributed to artefacts caused by the process of preparation of the sections or to be claimed as antemortem phenomena caused by the over-activity. The following circumstances speak explicitly for the latter; first, I got the same results in different kinds of preparations; second, I did not find any case of resting control fish, in which a similar picture of the reticulum was observed, and, third, I noticed a number of amoeboid glia cells with various catabolism products and the so-called 'Fuellkoerperchen. It must be emphasized here that the nuclei of the glia cells of the synapse showed not only regressive, but also progressive changes.

Buscaino (5), Rosental (31), and others studied the postmortem appearance of the amoeboid glia cells. Wohlwill (35) declared that through the postmortem decay of neuroglia tissue glia cells take occasionally the amoeboid appearance, and the occurrence of the methyl-blue granules and the 'Fuellkoerperchen' does not

always indicate the previous existence of amoeboid glia cells. In the present work all the fish brains, both normal and fatigue, were placed in the fixing solutions in their fresh condition, and the likelihood of postmortem production of the amoeboid glia cells cannot at all come under consideration. Attention must especially be called to the fact that in all my control preparations not a single amoeboid glia cell did come to my observation in and about the synapse.

What would then be the mechanism of the breaking up of the glia reticulum? It is very hard to answer this question definitely. Eisath (10), who studied and demonstrated the protoplasmatic glia structure very well by his own method, suggested that in the sections in which amoeboid glia cells occurred either in the marrow only or in both the marrow and the cortex, the glia cells with delicately arborized protoplasm processes do not come to observation. Alzheimer (1) also made the same observation; instead of the glia cells with arborized protoplasm processes, he found by means of the same method glia cells with a little increased plasm without any process or those with large cell body carrying different kinds of granules. He took for granted that with the appearance of the amoeboid glia cells the other glia structure also sustain some alteration. On another occasion Alzheimer (1) made the observation that in a cortex, which showed no glia cells with protoplasmatic processes by means of Mallory's method, the Golgi method revealed such cells with processes. On the ground of this finding, he carefully expressed his opinion, declaring that the processes did not here go into decay or were not withdrawn by the cells, but merely did not show the affinity to Mallory's hematoxylin.

Now, as already remarked, the processes of the glia cells of the synapse arborize and unite into a uniform glia reticulum in the synapse. This condition can be beautifully demonstrated in the Levaditi preparations. In fatigue a number of the glia cells are converted into the amoeboid glia cells and they lie free from the reticular structure of neuroglia tissue; and in this case attention must be called to the fact that some other glia cells in the synapse still show their relation to the reticulum and that I could observe

almost every stage of the dissolution of an amoeboid glia cell from the diffuse glia reticulum. Near the amoeboid glia cells the 'Fuellkoerperchen' or the fragments of the Golgi net beams were also observable, as already stated. So I came to the conclusion that in the extreme stage of fatigue a number of amoeboid glia cells are produced and are set free from the reticulum. Jakob (18) recently described in his article on secondary degeneration the detachment of the glia cells from the diffuse glia reticulum. Of course this process took place only slightly and on a small scale in my material, but the mechanism would here be the same. At the same time the substance of the reticular structure becomes looser, and finally some of the beams undergo dissolution and fall to pieces, so that the above-described alteration of the reticulum takes place. What effect the fixing solutions have here on the more or less loosened reticular beams, I can not say; but I believe that the mechanism of the breaking up of the glia reticulum could well be interpreted by the above-described facts.

As far as I know, the studies of the pathological alteration of the pericellular reticulum are very few; the studies of Eisath (10) and Alzheimer (1) were not especially directed toward the pericellular reticulum, but merely to the glia reticulum in general. Besta (4) investigated the behavior of the pericellular reticulum in certain pathological conditions, but his description does not explain the mechanism of the deterioration clearly enough and he did not mention the appearance of the glia cells at all. As far as the manifestation of the structure in question in fatigue is concerned, this investigation is unique.

THE BIOLOGICAL SIGNIFICANCE OF THE AMOEBOID GLIA CELLS AND THE CONCLUSION FROM THE RESULTS

As far as my observation went and so far as the histological technique used brought out the facts, over-activity caused no definite change in the nervous structure of the synapse of the Mauthner cell. Considering the nature of the experiment, one should not be surprised at this; moreover, the structures in question are so fine and the results of the technique for those structures are

sometimes so inconstant that one should be very careful to attribute any pathological significance to any slight histological manifestation in those structures. The appearance of the amoeboid glia cells may indicate, however, that some catabolism process takes place in and about the Mauthner cell. Alzheimer (1), who investigated thoroughly the amoeboid glia cells and the catabolism processes in the nerve tissue, said about the cases in which a number of amoeboid glia cells were found without any finding on the side of nervous structure, that some catabolism products which escape the microscopical demonstration at the present time would be produced on account of the disturbance of the nutrition of the nerve tissue. The finding of the amoeboid glia cells in the synapse might show that in over-activity some catabolism products are produced as the effect of pathological nutrition condition or owing to dilapidation of the nervous structure, not demonstrable at the present time. These would stimulate the formation of the amoeboid glia cells to serve as scavengers. That postmortem production of the amoeboid glia cells cannot come under consideration, I already remarked. Rosenthal (31), who wanted to interpret the appearance of amoeboid glia cells with methyl-blue granules as a sign of necrobiosis of neuroglia tissue, regarded the formation of the amoeboid glia cells with fuchsinophile granules as that of increased scavenging activity; the amoeboid cells with these granules were also found in fatigue, as remarked. According to Wohlwill (34), different kinds of diseases which show the amoeboid glia cells have edema as their common cause. The question whether over-activity causes edema in the region of the synapse and a swelling process of the glia cells can come under consideration or not, must be left undecided here. So far I may assume that in over-activity a catabolism process in a wide sense takes place in the synapse, which comes under the fourth category of catabolism processes of Alzheimer (2). But I cannot state definitely whether the catabolism products come only from the synapse or from both the synapse and the cell; the latter appears to me more probable.

The question whether the amoeboid glia cells come from newly produced glia cells or from those which were present in the synapse, is very hard to answer; but the finding of many amoeboid glia cells in spite of very little increase of the glia cells may indicate that at least some of the old glia cells give rise to amoeboid glia cells. As far as the transition of one kind of catabolism product into another within the protoplasm of the amoeboid glia cells is concerned, I cannot add much to the description of Alzheimer (1). The fact that we find the amoeboid glia cells with different granules and lipoid substance relatively more numerous around the blood-vessels than the other parts indicates that the catabolism products are assimilated into different granules by the amoeboid glia cells and carried to the blood-vessels and deposited in the cells of blood-vessels as fat and later are gradually disposed of.

In my experiments I could not find a single case in which the picture of the 'neuronophagia' of the Mauthner cell was observed; this might be interpreted to mean that the alteration of the cell body did not go so far in over-activity. Dolley (8) described cell death as the effect of over-activity; it is rather strange to me that no attention was directed to the changes of the glia cells in his article.

SUMMARY

Careful investigation of the cell body as well as the synapse of the fatigued Mauthner cell in Ameiurus revealed a number of interesting findings, which can be summarized as follows:

1. The cell body was found either swollen or shrunken; the turgescence was regarded as the result of over-activity and the shrinkage as that of exhaustion.

2. The Nissl substance was in a more or less advanced stage of chromatolysis and thereby the cytoplasm was stained variously deeply. On the border of the nucleus a mass of stainable substance was observed as the restitution phenomenon of the Nissl bodies on the side of the nucleus. It was also accepted that a mutual interchange of substance occurs between nucleus and protoplasm in activity.

3. The nucleus was also either swollen or shrunken; in the swollen nerve cell it was sometimes removed to one side of the cell body. The nucleolus was also found swollen sometimes and other times shrunken and it was of angular or otherwise irregular shape.

4. In the nervous structure of the synapse no definite alteration could be brought out; but the synapse showed a number of amoeboid glia cells with methyl-blue, fuchsinophil, and metachromatic basophile granules. Also fat drops were demonstrated in the glia cells and in the cells of the blood-vessels.

5. The reticular glia structure of the synapse appeared in many cases of fatigue in more or less advanced deviation from its normal configuration and even broken up in some parts; this was interpreted as the result of the detachment of the amoeboid glia cells from the reticulum, as also the effect of the loosening and dissolution of the net beams.

6. The appearance of the amoeboid glia cells showed that some catabolism process occurs in the synapse as the effect of pathological nutrition conditions in fatigue.

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Resumido por C. Judson Herrick, por el autor, O. Van der Stricht.

El desarrollo de las células de los pilares, el espacio del túnel y los espacios de Nuel del órgano de Corti.

El espacio del túnel se desarrolla alrededor del fascículo del nervio espiral, el cual camina entre las porciones nucleadas de las células internas y externas de los pilares. En su orígen es una hendidura intercelular cuyo contenido líguido es elaborado por el citoplasma vacuolar de las células de los pilares y se vierte en el espacio adyacente. Algunas partes de este protoplasma secretor sufren un proceso de citolisis, de tal modo que la hendidura crece y su contenido líquido aumenta en cantidad a sus expensas. El autor describe con detalle el desarrollo ulterior de las células de los pilares y sus cabezas. El primer espacio de Nuel aparece en forma de una hendidura longitudinal situada entre los pilares externos y las células ciliadas externas y en su interior se acumula el liquido segregado por los pilares externos. En las superficies laterales de estos últimos se proyectan vesículas de secrecióm claras, las cuales experimentan un proceso de citolisis y liquefacción. Las células externas de los pilares verifican na emigración embrionaria desde la primera fila de células ciliadas externas hacia las células internas de dichos pilares. El autor describe el desarrollo del segundo, tercero y cuarto espacio de Nuel. El contenido liquido del túnel y el del primer espacio de Nuel se mezclan a través de hendiduras existentes entre los pilares externos y comunican con las de los segundo, tercero y cuarto espacio de Nuel. El liquido de todos los espacios de Nuel está separado de la endolinfa del conducto coclear por los techos, muy delgados, de estos intersticios. Tales estructuras realizan, indudablemente, la propagación de las ondas vibratorias desde la membrana basilar hasta la membrana tectoria, contenida en el canal coclear.

Translation by José F. Nonidez
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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, APRIL 7

THE DEVELOPMENT OF THE PILLAR CELLS, TUNNEL SPACE, AND NUEL'S SPACES IN THE ORGAN OF CORTI

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EIGHTEEN FIGURES

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INTRODUCTION

In spite of numerous thorough and exhaustive investigations concerning the earliest stages of development of the organ of Corti, our knowledge of the origin of the tunnel space is still very limited and vague. This is true also of the formation of the heads and cephalic appendages of the pillar cells, and almost nothing is known concerning the origin of the so-called spaces of Nuel. This observer ('78) described in the organ of Corti in adult mammals a system of intercellular channels, and his findings have been confirmed by Retzius ('84) and other more recent authors. These spaces or channels are situated between

the outer rods of Corti and the neighboring outer hair cells, and between the three rows of outer acoustic elements. They contain fluid and are traversed by the phalanx processes of the cells of Deiters, intercommunicating through clefts between the sensory elements, and communicating with the tunnel space through interstices between the outer pillars. The view taken by Nuel, that they communicate with the lumen of the cochlea "par l'entremise de lacunes en forme de rames de la mambrane réticulaire," must be regarded as erroneous.

The development of the tunnel space between the two spiral rows of rods of Corti appears to be very difficult of observation. Indeed, most authors, referring to its appearance in embryonic material, state that it originates in the form of a narrow cleft between the inner and outer pillars, but give no details concerning the significance of this primitive interstice. Is it intercellular or intracellular? From what source is derived the fluid contained within the cleft? Those observers who clearly specify that the space appears between two neighboring pillars, as do Gottstein ('72), Retzius ('84), Vernieuwe ('05), N. Van der Stricht ('08), and Hardesty ('15), give no explanation as to the origin of its fluid. Alluding to the development of the space in rabbits two days after birth, Retzius states (p. 303):

> Von besonderer Bedeutung ist nun die enge Spalte, welche zwischen den beiden Pfeillerzellen reichen, ungefähr in der Mitte der Zellenhöhe, nach oben vom spiralen Nervenbündel entstanden ist und den Anfang des Tunnelraums darstellt; diese Spalte ist in der Basalwindung—wo indessen noch eine geringe Neigung der Pfeillerzellen nach aussen hin vorhanden ist—noch viel weiter entwickelt, und man sieht hier deutlich, dass die durch Einziehung (Verdünnung) der beiderseitigen Pfeillerzellen entstanden ist. Gleichzeitig ist aber auch die Anlage der Pfeiler in der Zellen als helleglänzende Streifen nunmehr wahrnehmbar. Nach aussen von der äusseren Pfeilerzellenreihe sieht man deutlich auch die Anlage der Nuelschen Raums.

According to Vernieuwe, the tunnel space is produced by the separation of the bases of the two pillar cells, due to elongation of the pillars, increase in size of the nuclei, and chiefly by the extension of the subjacent basilar membrane. Referring to the trend of the spiral organ of Corti towards the axis of the cochlea, Har-

desty ('15, p. 52) states: "The normal spaces between the elements of the spiral organ, including the large Nuel's space, no doubt result in part from this movement of the organ axisward." Other authors, Rosenburg ('68), Boettcher ('69), and Pritchard ('76) describe two neighboring inner and outer pillars as derived from a single original cell, the nucleus of which divides in two, and by a process of liquefaction of the undivided cytoplasm, the tunnel space is produced within it. This space is originally intracellular and its fluid is a protoplasmic product. Rickenbacher ('01, p. 402) seemingly ascribes a similar origin to the fluid of the space of Nuel in the adult guinea-pig: "Bei der Schnecke des ausgewachsenen Tieres hat der Prozess der Verflüssigung zur Bildung des Nuelschen Intercellularräume und des Leiterepithels geführt." According to Kishi ('02), the tunnel space is due to the spiral course of the nerve fibers after they have passed through interstices between the inner pillar cells. The formation of tunnel and intercellular clefts is considered by Held ('09) to be the result of 'ungleichen Wachstumbewegungen' of different epithelial cells. His so-called 'outer tunnel,' the spaces between the outer hair cells, and the space of Nuel outside the outer pillars are sheer intercellular channels, 'reine Intercellularspalten;' but the tunnel between the pillars is originally intracellular.

Eine reine intrazellular Spalt, da die ersten Nervenfasern, die hier spiralig abbiegen und weiter ziehen, nicht in der Zwischengrenze zwischen Aussen- und Innenpfeiler liegen, sondern im Protoplasma der Innenpfeilerzellen randständig eingebettet sind, was auch für die unten den inneren sowie äusseren Haarzellen resp. zwischen den Deiterschenzellen und in ihren Interzellularbrücken gelegenen Formation eines intraepithelialen Nervusplexus gilt.

The development of the tunnel and the pillar cells is closely connected with the formation of the pillar heads, the appearance of the 'head-plates' of the inner pillars, the phalanx processes of the outer pillars, and the extension of the membrana reticularis. The superficial structures of the rods of Corti in adult mammals have been exhaustively investigated by many observers: Max Schultze ('58), Koelliker ('59), Boettcher ('59, '72), Deiters ('60),

Hensen ('63, '71), Gottstein ('70, '72), Nuel ('78), Tafani ('84), Retzius ('84), and by most of the more recent authors; but the appearance and extension of these structures and the mechanical factors taking part in their formation require more careful study. N. Van der Stricht has shown that the head-plate of the inner pillar is originally represented by a very small square field, the apex of the cell, which becomes fibrillated and extends over the enlarging head of the outer pillar, the former undergoing great pressure from the latter The outer pillar cells originally belong to the first spiral row of outer sensory elements. As development advances they are pressed out from this row towards the inner rods of Corti and form a new row of outer rods, the apices of which always remain fixed between those of the outer acoustic elements of the first row. Hence there persists an apical segment of the outer pillar, which runs obliquely from the apex or phalanx of the cell, downward and inward toward the future head of the pillar. This oblique process contains a bundle of fibrils which, issuing from the head, passes between two outer acoustic elements and spreads out upon the phalanx—the head-plate of the outer pillar. By enlargement of the head, the fibrillar bundle gradually acquires a more horizontal position. Held ('09, p. 109) seemingly ascribes the head-plate of the inner pillar not only to the apex, but also to the superficial portion of the cell, "der obere Zellteil welche die Faserröhre enthält," and which is pressed flat from the developing head of the outer pillar. Although he did not recognize the original position of the outer pillar cells within the first row of outer acoustic elements, he nevertheless observes the squeezing of their 'Kopfplatte,' which becomes thinner from compression between two hair cells, and also of the bundle of fibrils, which at first run obliquely, then at right angles to the intermediate piece of the outer pillar, due to pressure from the elongating pillar cell.

In the present paper the appearance of the tunnel space, the development of the heads and cephalic appendages of the pillar cells, and the formation of the Nuel spaces will be dealt with in order.

DESCRIPTIVE

Appearance of the tunnel space

Sections tangential to the surface of the organ of Corti, and always somewhat oblique, affect transversely series of neighboring inner and outer pillars at various and successive levels of their length, from the superficial membrana reticularis toward the basilar membrane (fig. 1). As illustrated in figures 1 and 2, one may distinguish in the prismatic lamellar pillars three portions, although they are not sharply marked off: a basal or nucleated part, the largest, which is lamellar in shape or flattened out in a radial direction from mutual compression; an intermediate part; and a superficial part, the narrowest, which is compressed between the inner and outer hair cells, and hence more or less flattened out in a spiral direction (*ip* and *op*). The basal and intermediate portions are each made up of two cytoplasmic zones, the larger being clear and vacuolated, and occupying the area of the cell body close to the future tunnel; the smaller compact and fibrillated, and occupying the axial side of the inner pillar and the lateral side of the outer pillar. The superficial segment of the two rods of Corti contains no vacuolated protoplasm; it consists of a more homogenous, compact cytoplasm, which in the inner pillar is traversed by a bundle (fig. 2, *ip*) or a tubule (fig. 3, *ip*) of fibrils, and in the outer, encloses a bundle of fibrils which pass between neighboring outer hair cells and give rise to a small band, the phalanx process of the outer pillar (figs. 1, 2, and 3, *oph*), connected with the superficial apex of the cell, the phalanx. In the adult organ a part of this fibrillar bundle is a characteristic constituent of the superficial portion of the head, and thus its early presence in a definite portion of the outer pillar is very important in enabling one to determine, from the earliest stages of development, a very narrow but long superficial portion of the cell (figs. 1, 2, and 3, *op*), which later enlarges and becomes transformed into a part of the bulky head. It is also obvious that the adjoining portion of the inner pillar, which in figures 1, 2, and 3 is in close contact with this future head of the outer pillar, must be con-

sidered as the segment which will become converted into the so-called head of the inner pillar.

The outlines of all the pillars are very sharp, not only between the cells of the same row, but also between the neighboring elements of the two rows. While at the level of the superficial segments this outline is represented by an intercellular material (figs. 1 and 3, *tb*), which in its staining capacity and chemical constitution agrees with that of the superficial terminal bars, between the two lower segments of the pillars it is composed of a paler, more fluid, or true intercellular cement, in addition to which a very thin superficial cytoplasmic film can be brought into view. This outline and film are lacking along the axial surfaces of the inner pillars and the lateral surfaces of the outer. The spiral nerve bundle (N^{ii}) occupies an intercellular position between the nucleated parts of the outer and inner pillars, sometimes encroaching somewhat upon the lower interstice which separates their intermediate portions. The nuclei of the pillar cells are surrounded by vacuolated cytoplasm. The nuclei of the inner pillars are much smaller than those of the outer and much more flattened out radially.

When the tunnel space is about to appear, there occurs a characteristic alteration in the cytoplasm adjacent to this future cleft, the vacuoles running together and thus increasing in size (figs. 1 and 2, *t*). A common vacuolated mass soon appears (figs. 3 and 4, *t*); at certain places it remains fused with the cell body from which it is derived, at others it is independent, so that one cannot determine to which of the neighboring pillars it belongs. From this moment there exists a narrow intercellular cleft, filled with a small amount of extracellular, vacuolated material, a common mass which doubtless represents the first trace of the intratunnelar fluid, and which gradually increases in quantity by the coalescence of adjoining portions, partly incorporated in the original pillar and partly free or extracellular. Although from the earliest stages of the appearance of the space small extracellular, vacuolated masses can be found between the intermediate segments of the pillars (fig. 4, *t*), the larger part of the tunnel is generally seen around and close to the spiral

nerve bundle, that is to say, between the nucleated portions of the inner and outer rods of Corti (fig. 5, *t*, *T*), never "ungefähr in der Mitte des Zellenhöhe," as Retzius asserts and many investigators illustrate. It is a perinervous space. Later it extends between the intermediate portions of the pillar cells.

According to this description, the tunnel space must be held to be a true intercellular cleft, the fluid contents of which are developed by a process of secretion from the neighboring parts of the pillars and a simultaneous partial cytolysis of the latter. The space enlarges at the expense of the cell bodies. In the earliest stages of development of the organ of Corti there appears in the pillars not only a fibrillated sustentacular apparatus related to their function of support, but also a large, clear, vacuolated cytoplasm, the bulk, of their cell bodies. This portion of the protoplasm is glandular in nature, and from the blood plasma of the subjacent vas spirale (fig. 1, *vs*), it derives its nutritive material, which is elaborated and converted into clear vacuoles. The products of secretion are discharged along with a partial liquefaction of the surrounding cytoplasm.

During the extension of the tunnel space the superficial segments of the outer pillars undergo considerable enlargement, and their radial diameters soon correspond to those of the intermediate portions (fig. 4, *ohd*). At that time the process of cytolysis obviously extends along these segments (figs. 4 and 5, *t*), from below upward, involving a rapid reduction of their radial diameters. The intermediate segment of the outer and inner pillars, previously broad and formed of a small fibrillated part and a large vacuolated portion next to the future cleft, becomes gradually converted into a slender band, the so-called 'body' of the pillar. In figure 7 (*opb*) these bodies are shown cut at successive levels through fifteen outer pillars. In their lower portion, as seen in nine sections, they are reduced to thin cylindrical fibrillar strands, part of their apparatus of support, from around which the clear cytoplasm has disappeared. In their upper part, as seen in the next four sections, the pillar bodies are still composed of the original two zones, the vacuolated portion having been somewhat reduced. Close to the

future heads are seen two sections (connected with sections of inner pillars), the structures of which have undergone no change. The process of cytolysis is completed at the level of the first nine elements; it is progressing in the following four and has not yet begun in the last two. On comparing these structures with more advanced stages, and especially with those in the adult cochlea, it is plain that the body of the outer pillars acquires its final form and structure by a process of secretion and cytolysis along with the elongation of the intermediate segment. In young cats, bats, common and white rats it becomes a slender fibrillated strand, destitute of clear cytoplasm (figs. 10, 13^{v}, 14, 15, 17 and 18, *opb*). Between the pillar bodies, as already noted by Nuel, are large clefts through which the fluid of the tunnel space and the neighboring space of Nuel intercommunicate.

The intermediate portions of the inner pillars undergo similar, but never such marked changes. The greater part of their clear cytoplasm disappears, only a very narrow zone of it persisting, so that in young and adult animals the body of the pillar becomes lamellar in shape (fig. 18, *ipb*) and flattened out in a spiral direction. It is composed of a fibrillar lamella and a thin layer of clear protoplasm (fig. 17, *ipb*). Besides the pores traversed by the nerve fibers, no true intercellular clefts sever the inner pillars.

Along with these alterations and elongation of the pillar bodies, the tunnel space enlarges gradually but considerably, and very soon its radial diameter surpasses that of the two original clear zones belonging to two contiguous pillars. In other words, the fluid accumulated within the cleft exceeds the amount of disintegrated protoplasm. Indeed, the cytolysis occurs in a merocrine glandular cell, which, although undergoing partial liquefaction, is able to elaborate new clear secretion products at the expense of material derived from the vas spirale. Hence the tunnel fluid is the result not only of a sheer cytolysis, but also of a true elaboration and subsequent discharge. In the earliest stages of development the process of cytolysis seems to be more prevalent, since the contents of the cleft are seen in the form of a coagulated, vacuolated mass; afterwards the larger space is

usually filled with a clear uniform fluid, which seems to arise from a more active, true secretion. This secretion may continue even in the adult organ, for, according to all the investigators, the nucleus of each pillar cell is surrounded by a clear cytoplasm which extends over the floor of the tunnel. This protoplasm is vacuolated and represents the rest of the original bulky, glandular portion of both sustentacular and secreting cells.

Development of the heads and the cephalic appendages of the pillar cells

According to the results published in a previous paper (now in press) and those obtained by N. Van der Stricht, at the earliest stage of the development of the organ of Corti the outer pillar cells are located within the first spiral row of outer hair cells, and their superficial segments occupy interstices between two neighboring acoustic elements. By the rapid enlargement of the latter, these superficial elements are pressed out of the row and pushed towards the inner pillars, although their apices remain fixed between those of the hair cells. From this time the inner and outer rods of Corti constitute a scaffolding, which is made up of two spiral rows of sustentacular elements and is triangular in shape on vertical section. The rapidly enlarging base of the triangle abuts against the basilar membrane, and the apex is interpolated within the superficial membrana reticularis, separating the apices of the supporting and sensory elements of the inner spiral row from the apices of those of the first outer spiral row. In a section tangential to the organ of Corti the summit of the scaffolding is represented by a spiral row of very narrow fields, the apices of the inner rods of Corti, separated from one another and from the neighboring fields of the reticular membrane by deeply staining terminal bars, which extend into the depth between the superficial portions of the inner and outer pillar cells. Each of these narrow fields contains a diplosome, and will gradually enlarge by a process of compression from the underlying expanding heads of the outer pillars.

Outer pillars. In the superficial part of the entirely developed outer pillar, as seen in the adult organ of Corti, three different portions are distinguishable: 1) The apex or 'phalanx,' forming a part of the membrana reticularis. This consists of a lateral, expanded segment (fig. 8, *oph*^{i}), which constitutes a portion of the roof of a subjacent intercellular interstice, through which course the phalanx processes of the cells of Deiters of the first row (paper in press); and a medial, constricted segment (*oph*^{ii}) lying just between two apices of the outer hair cells of the first row (*oh*^{i}). 2) A fibrillated band or the phalanx process (fig. 13^{iii}, *oph*^{i}, *oph*^{ii}, *oph*^{iii}), which runs nearly horizontal and unites the apex to the head. 3) The head proper, or the enlarged superficial part of the pillar, in contact with the inner pillar (figs. 13^{iv}, 17, and 18, *ohd*). This is a cubical segment; in sections tangential to the surface it is square (fig. 13^{iii}, *ohd*) or somewhat lengthened out radially (fig. 18, *ohd*). Its upper portion is traversed by the fibers of the phalanx process (fig. 17, *ohd*), and its larger, lower part by a fibrillar bundle belonging to the body of the pillar (fig. 13^{iv}, *ohd*). Thus two different fibrillated fasciculi spread out and fade off into the head; there is no direct continuity between the fibrils of the two bundles (figs. 14 and 15, *ohd*).

In the first stage of development, which may last until the tunnel space is about to appear and before there is any marked increase in the site of the future head (figs. 1, 2, and 3, *op*), the three parts of an adult pillar just referred to are recognizable. The apex acquires the appearance illustrated in figure 4, *oph*. The phalanx process is short and a nearly vertical, deeply staining bundle of fibrils (figs. 1 and 3, *oph*) which is traceable between the cell bodies of the outer hair cells (*oh'*), and a little deeper between these sensory elements and the future head. The future head is a thin tapering part of the pillar, composed of a more or less homogeneous cytoplasm which encloses in its upper two-thirds the rootlets of the phalanx fibrils, and in its lower one-third the summit of the bunch of fibrils of the pillar body (figs. 2 and 3, *op*). Indeed, in figures 1, 2, and 3, two or three fields, cross-sections of the future head, contain parts of

the two fibrillated hands. This rather deep portion of the pillar, situated at the level of the lower poles of the outer hair cells (*oh*^{i}), doubtless belongs to the developing head. From this it is evident that the superficial, thin, tapering segment of the outer pillar cells, which gives rise to both the phalanx process and the head, attains more than one-third (figs. 1 and 3, *op*) or about one-half of the entire length of the cell, or about the length of the outer acoustic element (*oh*^{i}), although no distinct demarcation can be observed between the future head and the pillar body.

Two other features lend support to this view: the existence of an abundant, vacuolated cytoplasm along the intermediate portion of the cell, the future pillar body, which only slightly encroaches upon the lower part of the future head, and the presence of terminal bars or rather true intercellular, obturating partitions. These have been observed and termed 'bandelettes obturantes' by N. Van der Stricht ('08) and 'Kittsubstanz' or 'Kittlinie' by Held ('09). This material stains intensely with iron hematoxylin like the superficial terminal bars with which it is in continuity, and corresponds to them in nature and chemical constitution. It gives rise not to 'lines' or 'bars,' but to true septa, uniting parts of the cells and obturating the subjacent intercellular spaces. These partitions exist not only between contiguous developing and definitive heads of inner and outer pillars, but also between the apical surface (that turned toward the apex of the cochlea) and basal surface (that turned toward the base of the cochlea) of the heads of each spiral row. On the other hand, they are altogether lacking along the medial surfaces of the heads of the inner pillars and the lateral surfaces of those of the outer (figs. 1 and 3, *tb*).

The second stage of development is characterized by a rapid enlargement of the future head of the outer pillar (fig. 4, *ohd*), so that it reaches the site of the intermediate portion or even surpasses it, when the process of cytolysis progresses along the tunnel space (fig. 7, *op*). At first the head remains smaller next to the surface, but soon this portion expands and becomes somewhat larger than the deeper part (fig. 4, *ohd*) and acquires

a cubical or prismatic shape, the larger base of which touches the surface of the organ of Corti, its tapering apex blending with the much smaller pillar body. In cross-sections the prism is square or quadrangular in shape.

During this process of enlargement of the head, many remarkable changes occur. 1) A considerable shortening of the head segment (fig. 7) as if the compact substance of the lower parts had been pushed upward. Moreover, there can be no doubt that, at the same time, the vacuolated cytoplasmic zone of the intermediate portion of the pillar extends upward along the primitive head, so that the pillar body becomes longer at the expense of the latter. 2) A peculiar transformation of the protoplasm of the heads of the outer and inner pillars, close to and through the agency of the obturator septa. Primitively compact, homogeneous, or granular, entirely different from the vacuolated or fibrillated cytoplasm above referred to, the protoplasm of the head becomes converted into a denser material, staining intensely with iron hematoxylin. These changes occur in succession, first within the heads of the outer (figs. 4 and 7, *ohd*), then within those of the inner pillars (fig. 8, *ihd*), in proximity of the obturator septa separating their apical from their basal surfaces; later, along the medial surfaces of the heads of the outer pillars, and finally along the lateral surfaces of the heads of the inner pillars, close to the obturator partitions which separate these two elements (fig. 9, *ohd* and *ihd*). In sections tangential to the surface of the organ of Corti these altered cytoplasmic portions are seen in the form of deeply staining uniform, planoconvex masses, the planar surface of the clump of one head adjoining that of another mass belonging to a contiguous head (fig. 11). In reality, each planoconvex clump is the section of a vertical band or semicolumn. Thus in each head there appear three semicolumns, which at first are separated from one another, but in more advanced stages coalesce to form a single band or imperfect collar open toward the side of the head where the obturator material is lacking (figs. 7, 8, and 9). What mechanical factors cause these structures to appear is uncertain. It can only be stated that this dense and horny-

like exoplasmic head-collar develops and extends in close contact with the intercellular septa, as if the material elaborated at the periphery of the cytoplasm to increase the amount of extracellular cement were prevented from leaving the cell and retained within this collar, the staining capacity of which gradually increases, while the more central protoplasm, the endoplasm, becomes clearer and paler. This head-collar has been described in the embryonic pillar cells by N. Van der Stricht as 'plaque cuticulaire;' in the adult organ by Schwalbe ('87) as 'ellipsoider Einschlusskorper,' by Joseph ('00) and v. Spee ('01) as 'Kopfeinschluss,' and by Held ('02) as 'Kopfkörper.' 3) A change in the direction of the phalanx process and the intracephalic rootlets of its fibrils. Previously (fig. 1) inclined almost vertically, this fibrillar bundle gradually takes a more oblique course (fig. 3, *oph*), becoming in time nearly horizontal (figs. 4 and 6, *oph*) not only outside the head, but also within it, the fibrils occupying its superficial part. This alteration is caused doubtless by the shortening and considerable enlargement of the head and constitutes a striking evidence that this enlargement is the result not only of a sheer expansion, but also of a process of stretching of its lower parts in a more horizontal and radial direction, as if pushed upward by the strain of the elongating pillar body. At the same time, this pressure involves a conspicuous shortening of the previous cephalic segment. The peculiar change in the direction of the phalanx process has been observed by N. Van der Stricht and by Held ('09).

Inner pillars. In the adult organ of Corti the superficial portion of the inner pillar can be divided into three parts:

The apex, or 'Kopfplatte' of Held, the 'Innenpfeilerzellenschnabel' of v. Spee and Kolmer ('09), the 'plaque céphalique ou membrane fibrillaire' of N. Van der Stricht. This is a very thin, quadrilateral membrane (fig. 13^{i}), elongated radially and stretched between the apices of the sustentacular cells (originally the outer pillars) and the sensory cells (oh^{i}) of the first outer row and the apices of the supporting (is^{i}) and acoustic (*ih*) elements of the inner row of hair cells. It constitutes a part of

the membrana reticularis and is fibrillar in structure, the fibrils running parallel to the axis of the plate and in continuity with those of the head.

The so-called 'head' is formed of at least two segments, the smaller superficial one being in close contact with the head of the outer pillar. In the bat, the upper part appears to be reduced, from compression between neighboring elements, to a simple fibrillated lamella (fig. 13^{iii-iv}, *ihd*), while in the lower part there is a thin cytoplasmic layer lateral to the fibrils (fig. 13^{v}, *ihd*). In the white rat (fig. 17, *ihd*), the common rat (fig. 18, *ihd*), and particularly in the cat (fig. 9, *ihd*) twelve days after birth, this superficial lamella is obviously thicker, its lateral cytoplasmic layer being larger. In the bat (fig. 13^{v}, *ihd*) and other mammals this layer increases in breadth at the level of the lower part of the head, whence, without any demarcation, it blends with a larger, deeper segment. This is not connected with the outer pillar, but is situated below the head of the latter. It is a little shorter than the superficial segment and gradually tapers and continues with the body (*ipb*) of the pillar.

During the first stage of its development the future head of the inner pillar is a four-sided, somewhat flattened prism (figs. 1, 2, and 3, *ip*), nearly uniform in diameter, although tapering to its apex. It is composed of a granular or homogeneous cytoplasm and a bundle of fibrils, which occupy the medial side of the lower part of the prism and the central area of its superficial portion where, in the earliest stages of development, the fibrils are arranged in the form of a hollow tubule (fig. 3, *ip*) which later gives rise to a solid bundle. During the second stage of development the future head undergoes no very marked changes. By compression from the outer pillar head its superficial segment becomes somewhat thinner—lamellar in shape (figs. 4 and 6, *ip*)—while its lower segment maintains its previous size or enlarges slightly in the neighborhood of the pillar body. At the same time the transformations above mentioned are occurring in its cytoplasm in the proximity of the obturator septa. In order to clearly recognize the lamellar shape of the superficial segment of the head, cross-sections are needed. A longitudinal fibrilla-

tion as illustrated in figures 7 and 8 (*ihd*) indicates an oblique or more or less longitudinal section of the pillar, and such preparations are liable to misinterpretation.

The most remarkable changes occur at the level of the free apices of the inner pillars, the summit of the pillar scaffolding. The gradual development of the head of the outer pillar, situated just beneath this summit, produces a radial extension of the latter, and the transformation of a very small square field (figs. 1, 2, and 3, *aip*) into a long narrow fibrillated membrane or head-plate. This gradual extension is clearly shown in figures 4 (*aip*), 7 (*ipl*) and 8 (*ipl*^{i}, *ipl*^{ii}), whereas no enlargement in a spiral direction is noticeable. On measuring the radial diameters of the fibrillated head-plates in figures 3, 4, 7, and 8, and comparing them with the radial diameters of those portions of the membrana reticularis included between the plates and the outer border of the apices of the third row of acoustic elements, it is found that the former are respectively represented by about 1/11, 1/2.75, 1/2, and 1/1.64 of the latter. This statement gives a rather accurate picture of the rapid enlargement of the head of the outer pillar and the subsequent extension of the superficial inner pillar plate; that is, of the portion of the membrana reticularis formed by the latter during the development of the tunnel space.

From this description it is also evident, according to N. Van der Stricht (p. 610), that the extension of the apex of the inner pillar is due solely to a mechanical factor, a compression by the underlying enlarging head of the outer pillar. This view has been corroborated by Held ('09). He does not mention the description given by N. Van der Stricht but states (p. 212): "Je mehr der Kopf des Aussenpfeilers sich bildet und in seiner Masse wächst, um so dünner wird über ihm die Kopfplatte des Innenpfeilers." In its extension the head-plate undergoes important structural changes. Originally formed of a clear cytoplasmic field (figs. 1 and 3, *aip*) containing a diplosome or two central corpuscles, the elongating plate becomes subdivided into two zones, a lateral, small, clear zone, enclosing the diplosome (fig. 7), and a medial, more extensive, fibrillated one. This continues to lengthen and is composed of several parallel hori-

zontal fibrils, which, close to the apex of the inner hair cells, are continuous with the more vertical fibrils of the subjacent head lamella. Such structures depend upon the extension of the head-plate in a definite direction, *i.e.*, from a fixed point corresponding to the seat of the central corpuscle close to the outer hair cells, towards the inner acoustic elements.

Some sections tangential to the surface of the organ of Corti give pictures which prove that the head-plate is formed of two superposed planes, one deeper and fibrillated (figs. 8 and 9, *ipl*^{ii}), the other more superficial, destitute of fibrils, and composed only of a clear homogeneous cytoplasm (*ipl*^{i}), imperfectly enclosed by a part of the above-mentioned firm head-collar (fig. 8, *ipl*).

Structure of the heads of the inner and outer pillars in adult animals

The ultimate structural changes undergone by the heads of the pillars consist mainly in a broadening and extension of their collars. This band not only becomes thicker, but also extends over the head, to form the roof of the outer and inner pillar head (fig. 10, *ohd*, *ihd*). This roof appears to correspond to the 'plaque culticulaire' of N. Van der Stricht. Due to such transformation, the collar becomes converted into a head cap, a firm exoplasmic zone which circumscribes a clear granular endoplasmic zone, except at the medial side of the inner, and at the lateral side of the outer pillar. In other words, the remainder of the previous cytoplasm having now become much clearer, occupies a cephalic notch (fig. 18, *ohd*, *ihd*) which extends from the head roof towards the pillar body; the bottom and the lips of the groove are represented by the broadened head collar. The clear endoplasm filling up the notch is traversed by the fibrillar bundles of the heads, which in selected preparations stain deeply with iron hematoxylin.

The head of the inner pillar in the white rat (fig. 17, *ihd*) and in the common rat (fig. 18, *ihd*) is thus formed of a superficial thinner, and a deeper, enlarged segment, both composed of a medial groove containing a lamella of fibrils and a lateral layer of firm, dark, homogeneous cytoplasm—the walls of the notch.

This lateral layer enlarged rapidly toward the lower pole of the head and tapers downward to blend with a small uniform protoplasmic zone of the pillar body (fig. 17, *ipb*). In the cochlea of the adult bat, similar structures are seen (fig. 13^{v}, *ihd*), but near the surface of the head (fig. 13^{iii-iv}, *ihd*) only a very thin fibrillated lamella is recognizable. However, in vertical spiral sections showing the longitudinal fibrils throughout the length of the pillars, a part of the head cap is visible (fig. 16, *ihd*).

The notch of the outer pillar head enlarges from the roof towards the pillar body and presents a true asymmetrical position (figs. 17 and 18, *ohd*), and so is the structure of the head cap itself. On cross-sections the lips of the groove differ in thickness, the apical (i.e., that turned toward the apex of the cochlea) being obviously thinner than the basal (i.e., that turned toward the base of the cochlea). The clear cytoplasm of the notch is traversed by the nearly horizontal fibrils of the phalanx process (fig. 17, *ohd*), the rootlets of which merge obliquely into the apical lip. The other bundle of fibrils, running vertically from the pillar body toward the surface of the head, also shows an asymmetrical position. On passing into the head this bundle proves to be bipartite, being formed of a smaller and a larger fasciculus (fig. 13^{iv-v}, *ohd*). Within the lower and wider portion of the notch (fig. 13^{iv}, *ohd*) the subdivision into two unequal fasciculi is more evident, and the two bands are more closely connected respectively with the apical and basal lip of the groove. At the level of the head roof the horizontal fibrillated bundle courses through the cleft between the two vertical fasciculi, each of which merges into its neighboring lip (figs. 13^{iii} and 17, *ohd*). Most of these asymmetrical structures may be recognized during the development of the head (figs. 4 and 7, *op*). In vertical spiral sections of the adult organ, the asymmetry is very conspicuous. In figure 14 the apical surface (the surface turned toward the apex of the cochlea) of the head is clearly indicated by the course of the apical filament of the cells of Deiters (*ap*, d^{i}) in the direction of the apex of the cochlea. In such sections (figs. 14 and 15) can be seen the clear, eccentric oval notch, which contains a cross-section of the

horizontal bundle (*oph*^{iv}) and is outlined by a thinner apical border or wall, and a larger basal border, the bulk of the head. On penetrating into the head the fibrils of the pillar body (*opb*) become divided into two fasciculi, a thinner apical, and a broader basal one. The former seems to be shorter and its fibrils spread out obliquely through the corresponding lip; the latter is longer and its fibrils spread out fanlike (fig. 15, *ohd*) through the basal portion of the head, and seem to encroach upon the more homogeneous head roof. When the two systems of fibrils are not stained, the head roof can be more clearly seen to continue into the two borders of the notch. In the cochlea of young animals (fig. 10, *ohd*) the groove is much larger and its lips may be mistaken for sections through two different separate bodies, the 'ellipsoider Einschlusskörper' of Schwalbe and Joseph. These bodies do exist in earlier stages of development, but later, with the roof, they form one structure—the head cap.

The elongation of the phalanx process of the outer pillar will be dealt with in the next chapter.

The development of the spaces of Nuel.

With the exception of very short references, such as those alluded to above, no investigations have been carried out to determine the formation of the spaces of Nuel. Hence the problem appears to be a very knotty one and almost insolvable.

In the cochlea of adult animals the largest of these spaces is represented by a spiral cleft between the outer pillars and the cell bodies of the hair and supporting cells of the first outer row. This space may be termed the first space or the first spiral interstice of Nuel. Another cleft, which may attain considerable size, is the fourth space or spiral interstice of Nuel. This contains the phalanx processes of the cells of Deiters of the third row and is included between the hair cells of the third row and the so-called cells of Hensen. It is the 'external tunnel' of Held ('02). A second and a third space or spiral interstice of Nuel contain the phalanx processes of the cells of Deiters of the first and second rows, respectively, the

former situated between the outer acoustic elements of the first and second rows, the latter between those of the second and third rows. The second and third spaces do not extend between the long subjacent cell bodies of the supporting elements.

Appearance of the first spiral space of Nuel. This doubtless develops before the others and before any trace of the tunnel of Corti. The first trace of its appearance may be seen rarely (fig. 1) before the enlargement of the future heads of the outer pillars, in the form of clear, vacuolated, prominent vesicles on the lateral surfaces of the outer rods of Corti. How these vesicles are produced is uncertain; they seem to be only transitory and appear rather abruptly, as though due to pressure within the clear fluid contained in the vacuolated medial zone of the outer pillars, and as though part of this fluid had been driven across the outer fibrillated zone of the cell to give rise to large prominent vacuoles. These are seen along the lateral surfaces of the intermediate, the basal, and occasionally even parts of the superficial portions of the outer pillars. In more advanced stages their outlines and connections with the secreting cells become indistinct, and the vesicles are replaced by a common fluid mass, pervaded by a few delicate trabeculae in process of disintegration or liquefaction (fig. 3, *SN*). This process is not unlike that of cytolysis by which the fluid of the tunnel is produced. It is noteworthy that a distinct outline or a superficial membrane is never seen, either on the lateral surface of the outer pillars or on the medial surface of the inner pillars; so that under special conditions of intracellular pressure, fluid may exude and pass into intercellular channels. The cleft, filled up with this fluid, is the first space of Nuel. It enlarges gradually and extends toward the membrana basilaris, from which, even in the adult cochlea, it is separated by the lateral expansions of the feet of the outer pillars.

From this description it would appear that the initial dominant factor in the development of the cleft corresponds to a difference in pressure in two parts of the outer pillars: the large vacuolated medial zone, where clear fluid is being accumulated,

and the surface of the fibrillated zone, where a peculiar structure, the absence of a membrane, and a lower pressure, promote an exudation of fluid. In this respect a second important factor deserves due consideration, i.e., the shifting of the outer pillars. These structures originally are incorporated within the first row of outer hair cells, and although their extremities remain always fixed, their bodies are pushed inward and inside of the acoustic elements, so that at least a virtual, if not a true space appears below the first row of outer hair cells, between the nucleated portions of the cells of Deiters of the first row (fig. 1, d^1) and the outer pillars (*op*). This virtual cleft contains a spiral bundle of nerve fibers and represents the future space of Nuel.

The enlargement of the space of Nuel is doubtless promoted by a third peculiarity—a change in the shape of the outer pillar. When the shifting of the is latter completed, and before any appearance of a cleft, the lateral surface of the rod of Corti is a plane, represented in a vertical or oblique section by a straight line. Along with the lateral extension of the foot upon the basilar membrane (fig. 3, *op*) and the appearance of the head (figs. 4 and 7, *ohd*), and by a considerable elongation of the intermediate portion (the future body, fig. 7, *opb*), which is only possible by virtue of a curvation, the straight line becomes markedly curved, its concavity being turned towards and embracing the cleft. This may be a more important factor than appears at first sight. Indeed, in previous investigations (in press) it has been noted that the shifting of the cells of Deiters may be completed (that is to say, the sustentacular elements of the first outer row may be situated beneath their corresponding hair cells) before any appearance of a tunnel space (fig. 9 of the previous paper), or even of the true space of Nuel. In such figures, the original straight line persists, although the heads of the outer pillars are large, but the lateral extension of the feet is delayed.

Structure and transformation undergone by the phalanx processes of the outer pillars. Should any doubt be entertained as to the process of cytolysis along the lateral lower parts of the outer pillars, the structures and the transformation undergone by

their phalanx processes afford striking evidence of such a liquefaction. In the above description of these apical bands which unite the phalanges to the outer pillar heads, the most distinct constituent, the fibrillated bundle, alone has been mentioned. In the early stages of the development the band is composed of fibrils collected into a fasciculus, which is surrounded by a clear granular cytoplasm. Before the space of Nuel reaches the membrana reticularis this phalanx process proper is very short, being limited to the portion running between two neighboring hair cells (figs. 4, *oph*; 8 and 11, oph^{ii}), and the portion lying under the phalanx itself (figs. 8 and 11, oph^{ii}). In other words, the enlarged head contains the longest part of the fibrillar bundle (figs. 8, oph^{iv}; fig. 11, oph^{iii}, oph^{iv}) and covers completely the head plate of the inner pillar. The roof of the developing space of Nuel is made up of two strata, the lateral, thinnest part of the outer pillar head (fig. 11, oph^{iii}; compare with figs. 6 and 12), and the lateral part of the superficial striated membrane (fig. 8, ipl^{ii}). When the first interstice of Nuel has attained its entire extent in the adult organ its roof is composed of the lateral part of the head plates of the inner pillars (figs. 13^{i}, 17, and 18, ipl^{ii}) strengthened by equidistant, parallel, fibrillated bundles, portions of the ultimate phalanx processes (figs. 13^{iii}, 17, and 18, oph^{iii}), which run in an oblique direction toward the spiral rows of pillars (fig. 13^{ii}).

Figures 13^{i}, 13^{ii}, and 13^{iii} illustrate the structures of this roof at these successive levels in the adult organ of Corti. Between the apices of the inner hair cells (*ih*) and the outer sensory elements (oh^{i}) they show respectively a superficial plane—the striated head-plates of the inner pillar cells (fig. 13^{i}, ipl^{ii})—an intermediate plane composed of parts of the preceding plates (fig. 13^{ii}, ipl^{ii}) and parts of oblique subjacent fibrillar bundles, and a deeper plane (fig. 13^{iii}) showing from the axial to the lateral side, the row of fibrillated lamellae, heads of the inner pillars (*ihd*), the row of outer heads, a gap nearly as large as the preceding row and bridged across by equidistant fibrillar bundles (oph^{iii}), entirely devoid of clear cytoplasm. The gap is the upper floor of the space of Nuel (SN^{i}), which is

covered by the equidistant bundles and the lateral part of the uninterrupted striated membrane, formed of the head plates of the inner pillars. This description is corroborated by vertical spiral sections. In figures 14 and 15, above the heads of the outer pillars (*ohd*), is seen a dotted, very delicate membrane (ipl^{ii}), subdivided into short segments by coarser spots. This is the fibrillated membrane formed of the head-plates of the inner pillars; the fibrils have been cut transversely and the spots represent the sections of terminal bars which separate the plates. This dotted membrane extends over the neighboring space of Nuel (SN^{i}) and covers equidistant coarse granules (oph^{iii}), the cross-sections of the phalanx processes of the outer pillars. In figure 15 is seen, above the dotted line, a very fine, pale, uniform covering, which doubtless represents the homogeneous superficial zone (ipl^{i}) already mentioned.

The phalanx process of the outer pillar, represented in early stages by two portions, is formed in the adult cochlea of three segments, the original two—a subphalanx (fig. 13^{iii}, oph^{i}) and an intercellular segment (oph^{ii}) which courses between two hair cells—and a subsequently developed one, the submembranous stalk (oph^{iii}) which is derived from a part of the original intracephalic bundle (fig. 11, oph^{iii}). Indeed, transitional stages can be observed. In figure 11 an uninterrupted extracephalic clear protoplasmic layer, a kind of a pale veil, developed from the embryonic heads of the outer pillars (fig. 8, *ohd*) by a process of differentiation, unites three upper fasciculi (fig. 11, oph^{iii}) and assumes a festooned appearance around three lower bundles. This festooned appearance has been observed by N. Van der Stricht in the cochlea of a guinea-pig one day after birth. This investigator described (p. 641) each festoon as "une sorte de voile triangulaire à sommet dirigé vers la rangée des cellules acoustiques externes et à base en continuité avec la tête du pilier externe," and believes that the veil corresponds to the "Schwelling des Aussenpfeilerschnabels" of v. Spee. This clear protoplasmic sheath of the extracephalic bundle is seen also in vertical spiral sections (fig. 10, oph^{iii}). In the cochlea of a dog about four or five months of age it seems to persist,

but unquestionably disappears by a process of cytolysis in the adult bat (fig. 13^{iii}, 14 and 15, *oph*^{iii}) and the white rat (fig. 17). Evidences of such disintegration are shown in figures 9 and 10 (*cy*). The result is that a part of the clear cytoplasm which belongs to the originally enlarged heads of the outer pillars (fig. 8, *ohd*) undergoes a process of liquefaction. A portion of the intracephalic horizontal fibrillar band becomes free or at least partially destitute of protoplasm, so that the fluid of the large space of Nuel, in direct communication with that of the tunnel space through the wide interpillar clefts, comes in close contact with the very fine, superficial, fibrillated membrane of the head of the inner pillars. This membrane separates the fluid in question from that of the cochlea duct.

The physiological importance of these structures is evident, for the vibratory waves may be readily transmitted from one to another fluid through the intermedium of the striated membrane. As regards the transmission of vibrations from the fibrillated basement membrane of the membrana basilaris to the contents of the first interstice of Nuel, it is noteworthy that the former, at the level of the floors of the Nuel and tunnel's spaces, is separated from the latter by only a very thin cytoplasmic covering which belongs to the laterally expanded feet of the outer pillars and to the feet of the outer and inner pillars. Hence the transmission can be readily carried out.

Development of the second, third, and fourth spaces of Nuel. As shown in previous investigations, the phalanx processes of the cells of Deiters of the first and second rows are represented, in the earliest stage of development, by long apical segments of the cell bodies, which segments are included, respectively, in the second and third row of outer hair cells, within which they run between two neighboring acoustic elements. In length these apical segments agree with the hair cells. Due to the rapid enlargement of the latter, the apical segments are pressed out from their original row and shifted into interspaces; those of the first row of Deiters cells reaching the second future interstice of Nuel and those of the second row of Deiters cells reaching the third interval. All around the phalanx processes of the cells

of Deiters of the third row, which remain in situ between the third row of sensory elements and the cells of Hensen, will appear the large fourth interstice.

Before the appearance of any space the phalanx process is composed of a clear cytoplasmic sheath, enclosing a darker, axial, mitochondrial strand, which by juxtaposition and fusion of the chondrioconts becomes gradually transformed into an axial, fibrillated filament. The process is larger at its base, which issues from the nucleated cell body. and tapers to the superficial membrana reticularis.

In a kitten nine days old, at the level of the apical spiral turn of the cochlea, the second, third, and fourth sustentacular interstices are still filled up with the unmodified phalanx processes, so that intercellular spaces are absent. In the second turn, narrow channels appear and are somewhat larger near the surface of the epithelium than towards the base of the processes. Inversely the processes have become reduced in diameter at the expense of their clear protoplasm. At the level of the basal or third turn the enlargement of the spaces of Nuel and the reduction in size of the cytoplasmic sheath of the phalanx processes are much more marked. It must be noted that the thinning out of the latter is not the result of a sheer concomitant elongation, for these alterations are accompanied by a considerable elongation of the nucleated cell bodies of the sustentacular elements, involving a subsequent shortening of the supported hair cells, hence of the neighboring phalanx processes. These become more slender on account of a process of elaboration and secretion and a subsequent extrusion of clear fluid from the protoplasmic sheath. Whether, as many preparations seem to prove, this discharge is accompanied by a process of true cytolysis is uncertain, for these structures are very delicate and the shrinkage caused by the reagents might give rise to artefacts liable to misinterpretation.

The fourth space of Nuel develops in the same manner as the second and third, and when it appears, is but little larger than the others. It is occupied by the apical processes of the cells of Deiters of the third row. Originally as long as the neigh-

boring sensory elements, these processes are more numerous (previous paper) and larger than those of the first and second supporting rows. Situated outside the hair cells of the third row, they are not squeezed and impeded in their lateral expansion like the others. It is not to be wondered at that the products of secretion or cytoplasmic disintegration around the apical fibrillated bundles are more abundant and result in an expansion of the fourth interstice. Nevertheless, the process of development is identical with that of the two preceding spaces and therefore the application of a special term, external tunnel, to designate this formation is unnecessary. However, there can be no doubt that phalanx processes of the cells of Deiters of the third row retain their cytoplasmic sheath much longer than the others, and may show parts of it in the adult cochlea, as pointed out by Held ('02) for the 'apical type' of these cells in guinea-pig, cat, dog, and even the mouse. In such cases these processes are in closer connection with the outer wall of the space than with the medial.

In the basal spiral turn of the cochlea of a kitten twelve days after birth (fig. 12), the floor of the second, third, and fourth spaces of Nuel is formed by parts of segments of the cells of Deiters (d^{i}, d^{ii}, d^{iii}) supporting their corresponding sensory elements (oh^{i}, oh^{ii}, oh^{iii}). The medial and lateral boundaries of the second interstice are represented, respectively, by the lateral surfaces of the hair cells of the first row and the medial surfaces of those of the second. The medial and lateral boundaries of the third interstice are represented, respectively, by the lateral surfaces of the hair cells of the second row and the medial surfaces of those of the third. The adjoining surfaces of the acoustic elements of each sensory row are separated by narrow clefts, through which all of the spaces of Nuel intercommunicate. These channels, originally occupied by the phalanx processes of the sustentacular cells (those of the first sensory row being the superficial segments of the outer pillars), are liberated after the shifting of the phalanx processes. At first very narrow and virtually obliterated by the process of enlargement of the hair cells, these intercellular clefts become wider by the reduction in

size of the acoustic elements. The medial and lateral boundaries of the fourth interstice of Nuel are represented, respectively, by the lateral surfaces of the sensory elements of the third row and the medial surfaces of the so-called cells of Hensen, which, according to previous investigations, should be held as atrophied hair cells (aoh^{iv}).

The roofs of the spaces of Nuel are made up of parts of contiguous apices of the supporting elements (fig. 9). The roof of the second space is formed of alternating lateral and medial segments of phalanges of the outer pillar (aop), and the Deiters cells of the first row (d^{i}). The roof of the third space is composed of alternating lateral and medial segments of the phalanges of Deiters cells of the first (d^{i}) and second row (d^{ii}); the roof of the fourth space is represented by the lateral segments of the phalanges of Deiters cells of the second row and the apices of those of the third (d^{iii}). The roofs of the intercellular clefts between the hair cells of the first, second and third sensory rows are formed, respectively, of the medial or constricted part of the phalanges of the outer pillars (fig. 9, aop), the middle part of those of Deiters cells of the first row (d^{i}), and the middle part of those of Deiters cells of the second row (d^{ii}). The fluid contents of the first, second, third, and fourth spaces of Nuel intercommunicate through the intercellular clefts. Like the fluid of the first interstice, that of the others is separated from the contents of the cochlear duct only by very thin membranes, partially fibrillated, since the fibrillar bundles of the phalanx processes of the sustentacular elements spread over the under surface of their corresponding phalanx, according to the investigations of Held ('02) and of N. Van der Stricht. Such structures doubtless are able to promote the propagation of vibratory waves from the membrana basilaris to the fluid and the membrana tectoria within the cochlear canal.

SUMMARY

1. The tunnel space is developed around the spiral nerve bundle, which runs between the nucleated portions of the inner and outer pillar cells. It is originally an intercellular cleft, the fluid contents of which are elaborated in the vacuolated cytoplasm of the pillar cells and discharged into the adjoining space. Parts of this secreting protoplasm undergo a process of cytolysis or liquefaction, so that the cleft enlarges and the fluid contents increase in quantity at their expense.

2. The tunnel extends upward along the vacuolated zones of the intermediate portions of the pillars. This clear cytoplasm also disappears by a similar process of secretion and cytolysis. Ultimately the intermediate segments of the pillars become transformed into pillar bodies, reduced almost to their fibrillar apparatus of support, the outer being represented by thin cylindrical strands which are separated by large intercellular clefts, and the inner, lamellar in shape, being composed of a medial, thin, fibrillar lamella and a lateral narrow zone of clear cytoplasm.

3. In the earliest stage of development the heads of the outer rods of Corti are represented by thin tapering segments, nearly as long as the neighboring outer hair cells. They are characterized by the presence of two fibrillated bundles, the intracephalic rootlets of the fibrils of the phalanx process and the apical intracephalic extremities of those of the future pillar body. Moreover, peculiar structures, a more homogeneous cytoplasm and a system of obturator septa between the two rows of the future outer and inner heads and between the contiguous surfaces of the heads of each row, constitute important features, which enable one to recognize the nature of these apical segments.

4. In a more advanced stage the long superficial segment of the outer pillar enlarges rapidly into a shorter prismatic mass, the head proper, within which develop, in contact with the obturator septa, a firmer exoplasmic collar, and ultimately a head cap, enclosing imperfectly a clearer endoplasmic mass, which is traversed by the nearly horizontal and the vertical

fibrillated bundles, the rootlets of the fibrils of the phalanx process and the apical ends of those of the pillar body, respectively.

5. The original head of the inner pillar is represented by a small, four-sided, somewhat flattened prism. Later by compression from the outer pillar's head, the superficial part of the prism assumes a more distinctly lamellar shape; the lower part enlarges and acquires its largest size at the level of the lower pole of the outer pillar's head, whence it tapers upward and downward. The so-called 'head' of the inner pillar extends down beyond that of the outer and furnishes a pad of support to the latter. The prismatic head is composed of a lateral uniform cytoplasm, which enlarges at the level of the lower, broader portion, and a medial fibrillated lamella. In contact with the obturator septa and within the homogeneous protoplasm the head cŏllar develops.

6. By compression from the underlying and enlarging head of the outer pillar, the free apex of the inner pillar undergoes a gradual and extensive elongation, becoming converted into a fibrillated, long head-plate. The constant position of the central corpuscle within this mèmbrane, close to the apices of the outer hair cells of the first row, proves that this elongation occurs in a definite direction, from the seat of the diplosome toward the apices of the inner hair cells.

7. The heads of the outer pillars are asymmetrical in structure. The cephalic notch, imperfectly surrounded by the head cap, is traversed by two fibrillated bundles and has a parapical position; it is situated nearer to the apical surface of the head (i.e., the surface turned toward the apex of the cochlea) than to the basal, since the apical lip of the groove is thinner than the basal. The horizontal rootlets of the fibrillated phalanx bundle are also parapical and merge obliquely into the apical border of the notch. The vertical fibrillar cephalic bundle, arising from the pillar body, divides into two unequal fasciculi, a smaller and a larger, circumscribing a cleft through which run the horizontal fibrils. The smaller fasciculus merges into the apical lip of the notch and the larger one into the basal lip, the bulk of the head cap.

8. The first space of Nuel, which, in the adult organ of Corti, is situated between the outer pillars and the outer hair cells and the cell bodies of the sustentacular elements of the first row, appears as an intercellular cleft, within which is accumulated a fluid discharge from the outer pillars. At the lateral surfaces of the latter project clear secretion vesicles, which undergo a process of cytolysis and liquefaction. The exudation of this fluid seems to be due to a difference of pressure, on the one hand within the medial vacuolated cytoplasmic zone of the outer rods of Corti, and on the other, at their lateral surface. It is promoted by the shifting of the embyronic pillars from the first spiral row of outer hair cells towards the inner rods of Corti, by the development of laterally enlarged segments at the two extremities of the outer pillars, the foot and the head, and an elongation of the outer pillar bodies, only possible by virtue of an incurvation, the concavity being turned towards the cleft of Nuel.

9. This process of cytolysis is manifest at the level of the heads of the outer pillars. Indeed the enlarged embryonic head is more bulky than the adult head, and the phalanx process, shorter in the earlier stages of development, later becomes longer. Originally the phalanx process is represented by two segments, a subphalangeal and an intercellular segment, the latter running between two hair cells. Later on, a submembranous segment appears. This lies beneath the lateral portion of the head plates of the inner pillars, and is developed from a lateral part of the enlarged outer head by a process of disintegration of clear cytoplasm, which encloses the horizontal intracephalic fibrillated bundle.

10. The roof of the first space of Nuel in the earliest stages of development of this interstice is composed of two uninterrupted coverings, one superficial and very thin, the lateral portions of the head-plates of the inner pillars, the other deeper and much thicker, the lateral parts of the outer heads. In the adult cochlea this roof is made up of the same parts of the superficial head-plates and a largely interrupted covering, the equidistant submembranous segments of the phalanx processes.

11. The second, third, and fourth spaces of Nuel are located, respectively, between the first and second, the second and third, and the third row of hair cells and the cells of Hensen (atrophied hair cells of a fourth row). These spaces do not extend down between the sustentacular elements, but communicate with each other and with the first space through clefts between the hair cells. These intercellular channels, originally occupied by the phalanx processes of the sustentacular elements, become free after the shifting of the latter into the neighboring medial spaces, the phalanx processes of the cells of Deiters of the third row remaining in situ.

12. Each of these phalanx processes is composed of an axial, fibrillar filament and a peripheral, clear, cytoplasmic sheath. In the course of development this sheath becomes thinner and may disappear by a process of secretion, which gives rise to the fluid contents of the primitive second, third and fourth spaces of Nuel.

13. The roofs of the second, third, and fourth spaces of Nuel and of the intercellular clefts between two neighboring hair cells of each sensory row are made up of delicate membranes, partially fibrillated, which belong to various parts of the phalanges of the sustentacular elements.

14. The fluid contents of the tunnel and the first space of Nuel are separated from the fibrillated basement membrane of the membrana basilaris by a thin protoplasmic covering, belonging to the feet of the inner and outer pillar cells. They intercommunicate through clefts between the outer pillars and communicate with those of the second, third, and fourth spaces of Nuel. The fluid of all the spaces of Nuel is separated from the endolymph of the cochlea duct by the roofs of these interstices, very thin membranes, entirely or partially fibrillated. Such structures doubtless promote the propagation of vibratory waves from the basilar membrane to the membrana tectoria, contained in the cochlear canal.

All the material and reagents necessary for the present investigations were supplied by Dr. T. Wingate Todd, Director of the

Anatomical Laboratory of the Medical School, Western Reserve University, Cleveland, Ohio. It affords the author great pleasure to express his deep gratitude to Dr. Todd.

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DESCRIPTION OF PLATES

All figures were outlined with a Zeiss camera lucida, at the level of the stage of the microscope, with the aid of a Zeiss ocular no. 3, and 2-mm., homog. immersion. Apert. 1.30, except figures 1, 2, 3, 4, 5, and 7, which were outlined with Zeiss ocular no. 1.

GENERAL ABBREVIATIONS

aip, apices of embryonic inner pillar cells

aoh^{iv}, apices or bodies of atrophied hair cells of an outer fourth spiral row

aop, apices or phalanges of the outer pillar cells

cy, cytoplasm in process of cytolysis

d^{i}, *d*^{ii}, *d*^{iii}, apices or bodies of cells of Deiters, respectively, of the first, second, and third rows

H, apex of the cell of Hensen

ih, apices or cell bodies of the inner hair cells

ihd, heads of the inner pillar cells

ip, inner pillars

ipb, bodies of the inner pillars

ipl, head-plates of the inner pillars

ipl^{i}, *ipl*^{ii}, respectively, the superficial homogeneous zone and the deeper fibrillated zone of the head plates of the inner pillars

is^{i}, *is*^{ii}, apices or cell bodies of supporting cells, respectively, of the first and second inner rows

N, Nerve bundle passing through the foramen nervinum

N^{ii}, spiral nerve bundle running between the inner and outer pillar cells or within the tunnel space

N^{iii}, spiral nerve bundle running between the outer pillars and the cells of Deiters of the first row

nd, apices of non-differentiated cells of the greater epithelial ridge

ni, nuclei of non-differentiated cells of the greater epithelial ridge

nih, nuclei of inner hair cells

nip, nuclei of inner pillar cells

nis^{i}, *nis*^{ii}, nuclei of inner supporting cells, respectively, of the first and second rows

nop, nucleus of an outer pillar cell, seated near the head (abnormality)

oh^{i}, *oh*^{ii}, *oh*^{iii}, apices or cell bodies of outer hair cells, respectively, of the first, second, and third rows

ohd, heads of outer pillars

op, outer pillar cells

opb, bodies of outer pillars

oph, phalanx processes of outer pillars

oph^{i}, *opd*^{ii}, *opd*^{iii}, *opd*^{iv}, respectively, the subphalanx, intercellular, submembraneous segments and the intracephalic roots of the phalanx process of the outer pillar

pd^{i}, *pd*^{ii}, *pd*^{iii}, phalanx processes or apical filaments of cells of Deiters, respectively, of the first, second, and third rows

SN, *SN*^{i}, the first space of Nuel

t, developing tunnel space

T, tunnel space

tb, terminal bars or obturator septa

VS, vas spirale

PLATE 1

EXPLANATION OF FIGURES

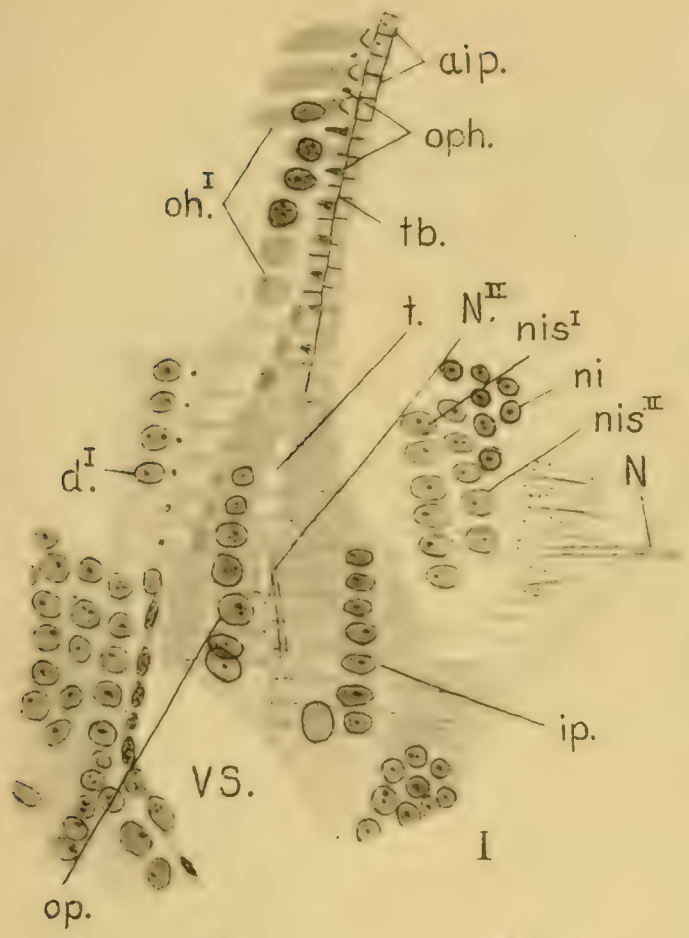
1 Section tangential (and somewhat oblique) to the surface of the organ of Corti, through the second (middle) turn of the cochlea. New-born kitten. Fixation: osmic acid, 1 per cent aqueous solution for about one hour, followed by immersion in Zenker's fluid. Stain: Iron hematoxylin, Congo red, light green.

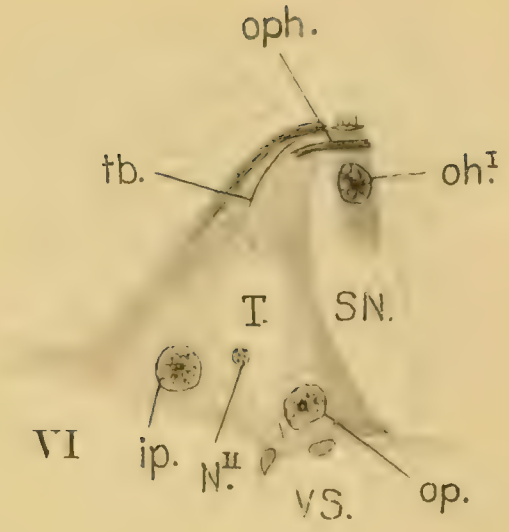
2 Section tangential to the surface of the organ of Corti, through the second turn of the cochlea. Kitten 3 days, 12 hours after birth. Exposure of the cochlea, the bony wall of which had previously been provided with two small openings, to vapors from a 2 per cent aqueous solution of osmic acid for approximately one hour, and subsequent treatment of the piece by trichloracetic acid 5 per cent in water. Iron hematoxylin, Congo red.

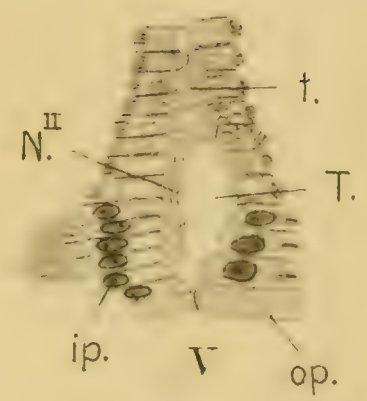
3 Section tangential to the surface of the organ of Corti, through the basal portion of the second turn of the cochlea. Dog 3 days, 18 hours after birth. Zenker's fluid. Iron hematoxylin, Congo red.

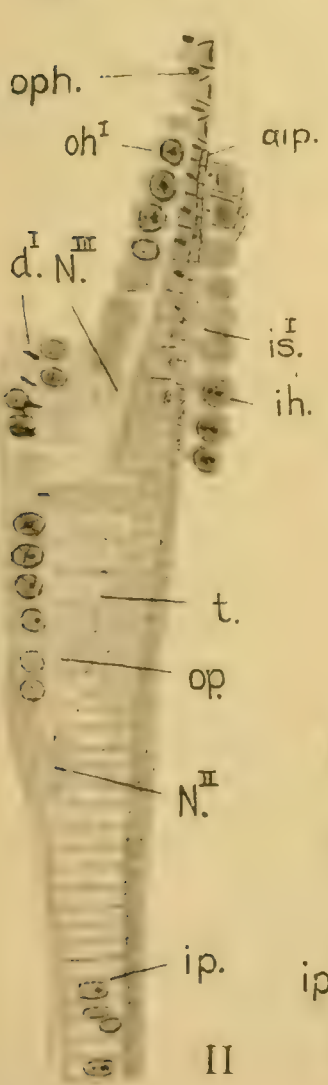
4 and 5 Sections tangential to the surface of the organ of Corti, through the basal portion of the second turn of the cochlea. Kitten 3 days after birth. Solution of trichloracetic acid 5 per cent in water. Iron hematoxylin, Congo red, light green.

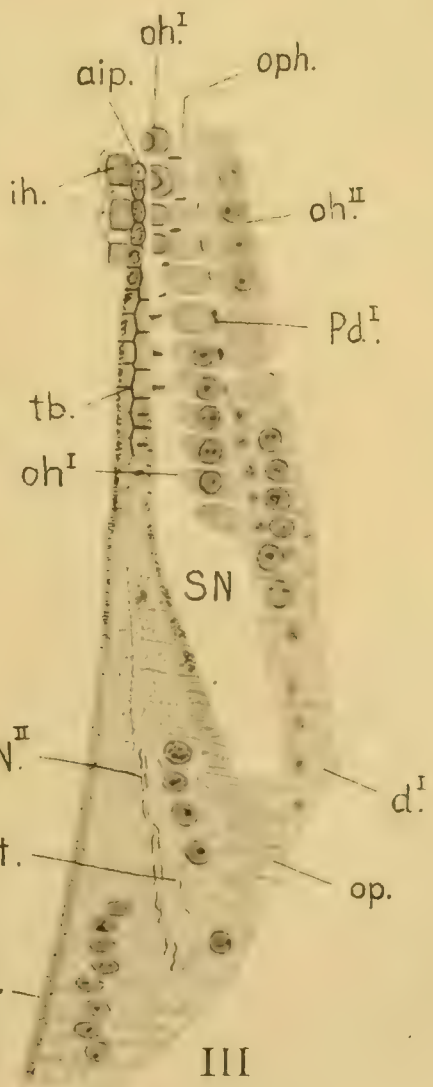
6 Radial vertical section of the organ of Corti through the third (basal) turn of the cochlea. Kitten 3 days, 12 hours after birth. Exposure of the cochlea to vapors from a 2 per cent aqueous solution of osmic acid and subsequent treatment of the piece by trichloracetic acid 5 per cent in water. Iron hematoxylin, Congo red.

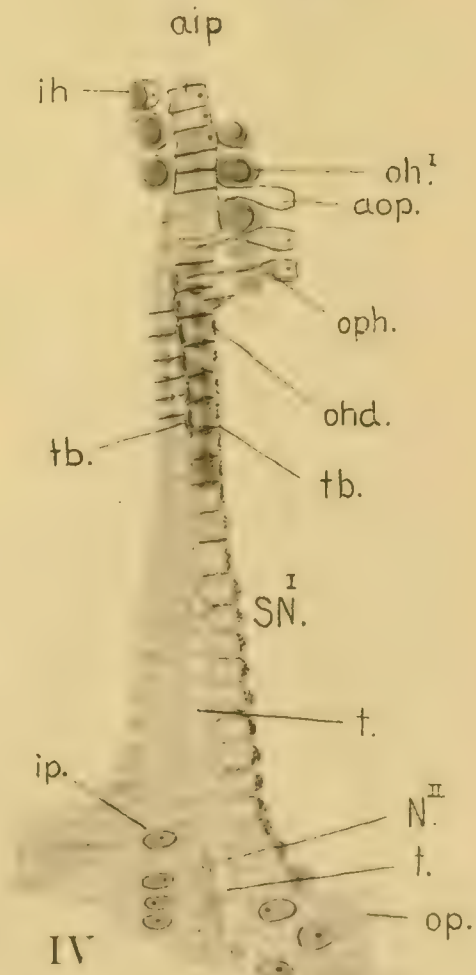












O. Van Der Stricht fecit

PLATE 2

EXPLANATION OF FIGURES

7 Section tangential to the surface of the organ of Corti, through the third turn of the cochlea. Dog 3 days, 18 hours after birth. Solution of trichloracetic acid 5 per cent in water. Iron hematoxylin, Congo red.

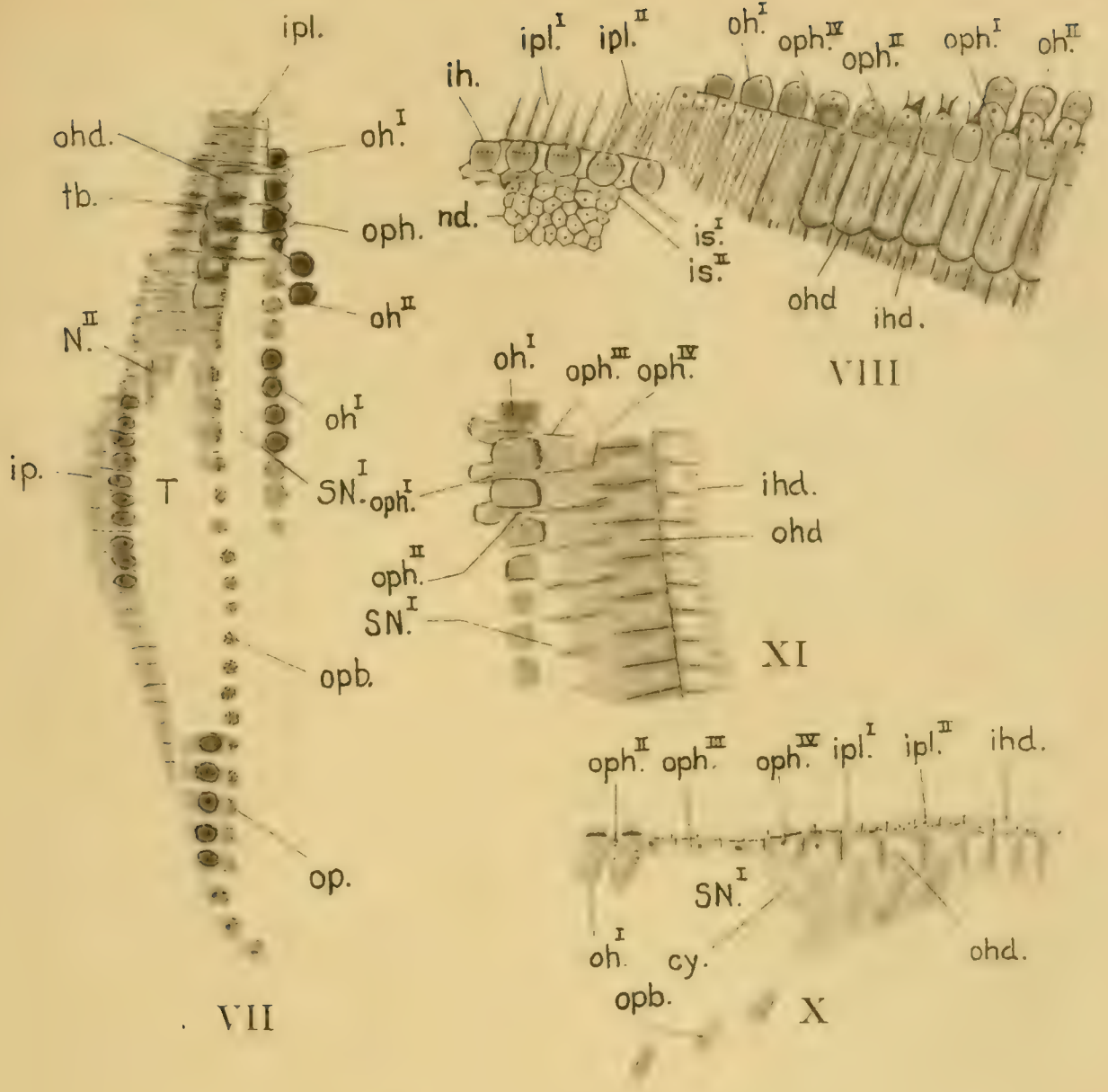
8 Section tangential to the surface of the organ of Corti, through the third turn of the cochlea. Kitten 5 days, 12 hours after birth. Solution of trichloracetic acid 5 per cent in water. Iron hematoxylin, Congo red, light green.

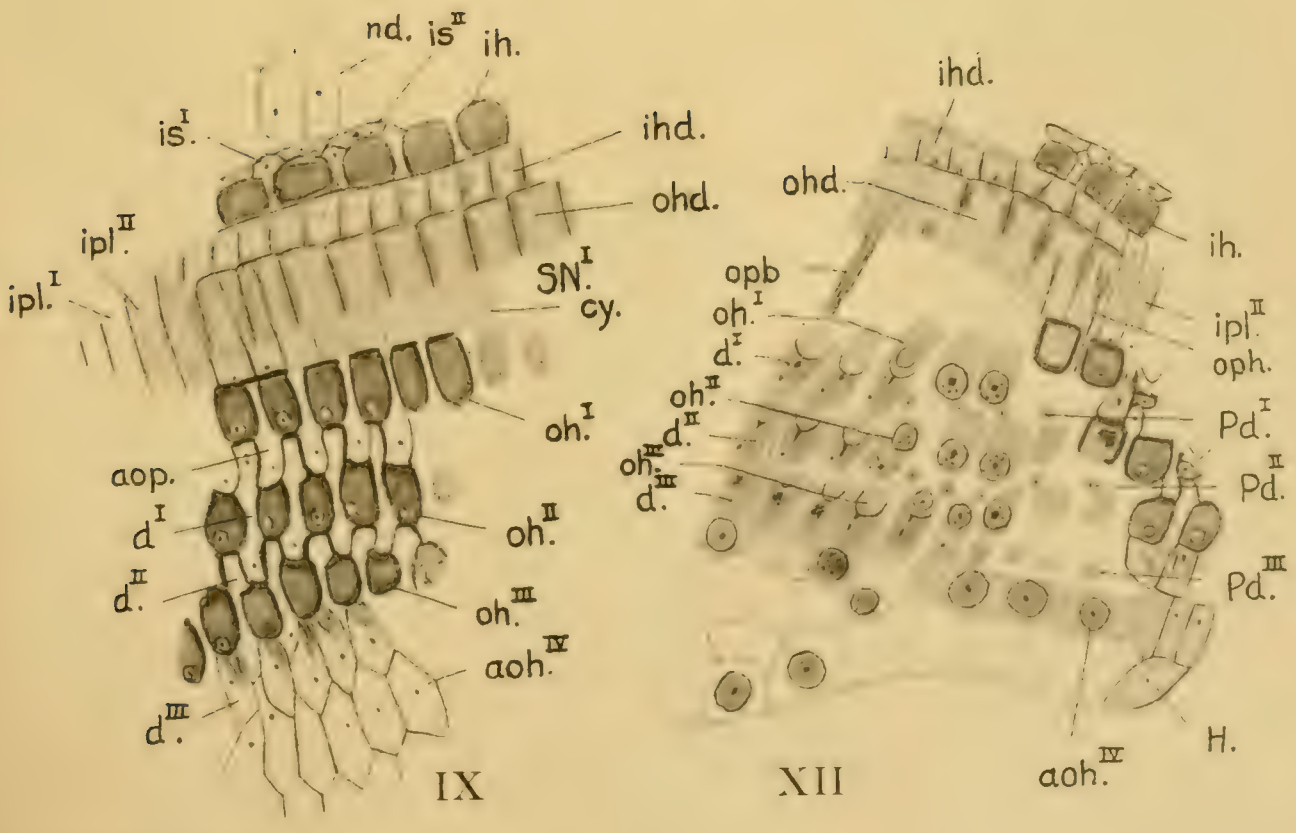
9 Section tangential to the surface of the organ of Corti, through the basal portion of the first (apical) turn of the cochlea. Kitten 12 days after birth. Osmic acid 1 per cent aqueous solution for about one hour, followed by immersion in a 5 per cent aqueous solution of trichloracetic acid. Iron hematoxylin, Congo red.

10 Vertical spiral (parallel with the spiral rows) section of the organ of Corti, through the second turn of the cochlea. Kitten 11 days after birth. Osmic acid 1 per cent aqueous solution for about one hour, followed by immersion in a 5 per cent aqueous solution of trichloracetic acid. Iron hematoxylin, Congo red.

11 Section tangential to the surface of the organ of Corti, through the basal portion of the second turn of the cochlea. Kitten 12 days after birth. Osmic acid 1 per cent aqueous solution for about half an hour, followed by immersion in a 5 per cent aqueous solution of trichloracetic acid. Iron hematoxylin, Congo red, light green.

12 Section tangential to the surface of the organ of Corti, through the third turn of the cochlea. Kitten 12 days after birth. Osmic acid 1 per cent aqueous solution for about half an hour, followed by immersion in a 5 per cent aqueous solution of trichloracetic acid. Iron hematoxylin, Congo red.





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PLATE 3

EXPLANATION OF FIGURES

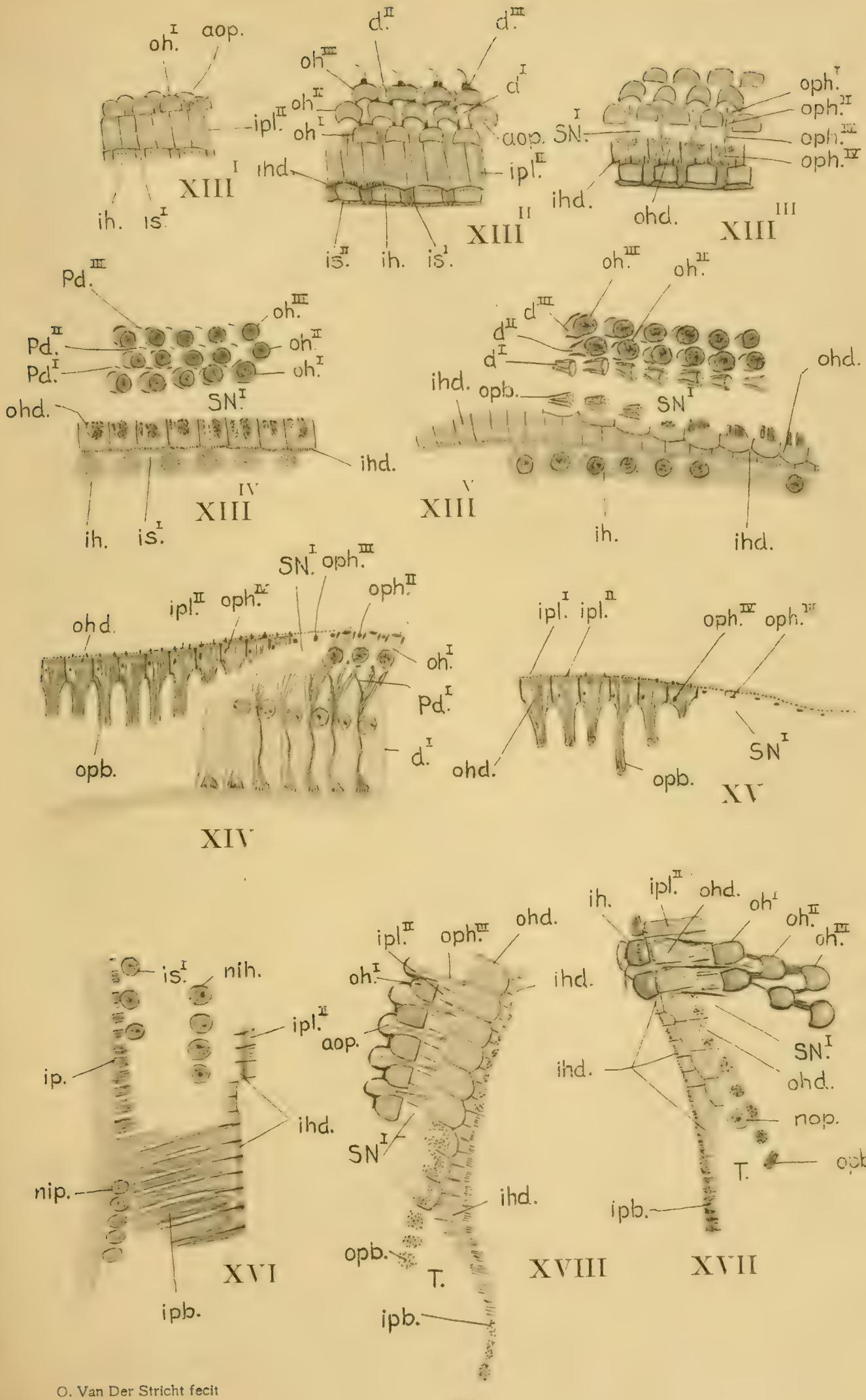
13^{i-v} Sections tangential to the surface of the organ of Corti through the third turn of the cochlea. Adult bat (Vespertilio fuscus). Zenker's fluid. Iron hematoxylin, Congo red. The figures illustrate structures at five successive levels of the organ of Corti.

14 and 15 Vertical spiral sections of the organ of Corti, through the second turn of the cochlea. Adult bat (Pipistrellus subflavus). Bouin's fluid. Iron hematoxylin, Congo red, light green.

16 Vertical spiral section of the organ of Corti through the second turn of the cochlea. Adult bat (Pipistrellus subflavus). Trichloracetic acid. Iron hematoxylin, Congo red, light green.

17 Section tangential to the surface of the organ of Corti, through the second turn of the cochlea. Adult white rat. Trichloracetic acid. Iron hematoxylin, Congo red, light green.

18 Section tangential to the surface of the organ of Corti, through the third turn of the cochlea. Adult rat (Mus decumanus). Bouin's fluid. Iron hematoxylin, Congo red, light green.



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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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VERTEBRATE CEPHALOGENESIS

IV. TRANSFORMATION OF THE ANTERIOR END OF THE HEAD, RESULTING IN THE FORMATION OF THE 'NOSE'

HOWARD AYERS

TWENTY-SIX FIGURES

CONTENTS

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INTRODUCTORY

From the investigations of many anatomists we have come to recognize, first, that the jaw apparatus (including the tongue and mouth) is a mechanism built up out of old-time head cartilages and newer elements derived from the gills, which has been added to the primitive vertebrate head—in fact, a mechanism of the trigeminus. Second, that the whole auditory apparatus is a mechanism built up out of surface sense organs sunk below the surface, together with structures derived from the gills. Third, that the eyes and adnexa are the end result of the outpushing from the brain of two hollow globes, whose walls are made up of the pigmented light sensitive cells of that part of the central nervous axis behind the lamina terminalis, with numerous associated parts. These three prominent organ systems have been quite thoroughly worked out and their component parts traced back to their origins. In speaking thus of the evolution of these three prominent additions to the head, we include those other necessary structures, as blood, nerve and lymph supply, and the muscles, connective-tissue parts and skin, which go to correlate

and cover these organ systems. As regards the nose, it is generally agreed that the olfactive organs are derived from a pair of sensory organs, formed near the anterior end of the head which very early sink below the surface of the skin of the snout, later to become housed in a cavity called the nasal chamber. The details of this process and the structures involved have not been definitely worked out and described.

The following condensed account of some of the results of my study of the vertebrate nose has for its object, making clear the manner in which the human nose has come to be and also to homologize the several structures entering into its formation. The literature is extensive and regarding such structures as, e.g., Jacobson's organ, the N. terminalis, and the vomeronasal nerve, by no means harmonious either in statement of fact or interpretation. No attempt will be made here to review the literature, it being considered more important to lay the foundation for a rationals tudy of the nose and nasal region of the vertebrate head.

Beginning with the stage of head development presented by Amphioxus, we pass to Ammocoetes, Petromyzon, Bdellostoma, and Man. Already in the Marsipobranchs, the main features of human nasal anatomy are laid down, since the nasal chamber of Bdellostoma contains the terminal organs supplied by three pairs of cranial nerves with the endings of the invading branches of the trigeminus.

1. AMPHIOXUS

The anterior end of the head region of Amphioxus (figs. 1 and 12) is compressed from side to side and has the shape of a spearhead, viewed either from the side or above. Included within it we note the anterior end of the central nervous system with the terminal paired but unseparated eye rudiments and the primitive olfactive organs. This part of the nervous axis contains the ventricular cavity and is the earliest stage known to us of the vertebrate brain. Connected with the brain from before, backward, are the following paired nerves (figs. 1, 2, 3, 4, 5, 6, 7, and 12): 1. N. terminalis. 2. The N. opticus which does not extend beyond the brain contour. 3. N. olfactorius. 4. N. septalis.

The N. terminalis[1] is connected to the right and left side of the anterior end of the ventral plate of the brain, and owing to the relatively large size of this pair of nerves they appear, when viewed from above, like a bifurcation of the brain. They run forward to the tip of the head. The N. opticus is entirely imbedded in the front wall of the brain anterior to the ventricle. In Amphioxus we have a stage of the evolution of the eye antedating the formation of optic cups which, from Bdellostoma on, presents such a prominent feature of vertebrate anatomy. The N. olfactorius (figs. 2, 3, 4, and 7), being relatively small, is a short nerve which arises from the anterodorsal wall of the brain near to the median line and, while the right and left olfactorius arise from the right and left halves of the brain respectively, they are usually drawn close together into one trunk as they approach the olfactive organ, although they occasionally remain separate and distinct their entire length. They run dorsad and cephalad and innervate the right and left halves of the olfactive cup just as their homologues do in Ammocoetes and all other vertebrates. The N. septalis arises from the dorsal wall of the brain on either side of the median line above the posterior limit of the ventricular cavity. The two nerves curve upward, forward, and outward, and run to the sides of the anterior end of the head, innervating the territory mainly caudad of the N. terminalis and as far back as the hypophysis.

In Amphioxus the body surfaces innervated by the terminal and septal nerves (figs. 1 and 12) are fully exposed, except the hypophysial region which lies within the buccal cavity; with this exception they form part of the body contour. The olfactory organs are sunk below the body surface as a conical pit opening directly on the surface and more or less pushed to the left of the median line by the dorsal head fin fold.[2]

The distribution of the septalis nerve in Amphioxus is as follows: Arising from the dorsolateral territory of the brain above the posterior border of the ventricle the nerve trunk soon separates (figs. 1, 12 and 26) into two parts. The larger part curves forward and outward, dividing as it passes to its terminal territory behind the tip of the snout which is supplied by the N.

terminalis. The smaller branch curves forward and downward over the surface of the notochord and innervates the surface territory about the anterior end of the buccal cavity as well as the walls of the terminal pocket of the mouth and the dorsal hypophysial organ which formerly occupied a surface position as the preoral pit of the larva.

The terminal territory of the Amphioxus body is thus a terminal sensory organ. As far as known, the sensory elements are isolated sensory cells distributed with some regularity of spacing throughout the epithelial covering of the body in the territory supplied by the terminal and septal nerves. These sensory cells have not been found in other localities. They are innervated by terminal twigs given off from the short fibrils which issue from the groups of subepithelial ganglion cells belonging to these two nerves (fig. 11). The close association of the terminal and septal nerves in their peripheral distribution is reflected in the central exchange of fibers (figs. 8, 9, and 10) and doubtless in their physiological functions, to the extent of their being grouped to-

ABBREVIATIONS

A, place of origin of the hypophysial organ
B, last surface position of hypophysial organ
Br, brain.
C, position of hypophysial organ in adult
G, nasal gland (Jacobson's) organ
H, hypophysis
HC, hypophysial canal
h, hypophysial extension
H.S., hypophysial sac
L.O, lobus olfactorius
l.t, lamina terminalis
M, mouth
M', anterior buccal pouch
N, nose
n, external nasal opening
n.f, nasal fold
n.n, nasal nerve
NC, nasal canal
NF, nasal fold
N.p, nasopalatine nerve
O, olfactory nerve
OL, *L.O*, olfactory lobe
ON, olfactory nerve
op, optic nerve
P, anterior buccal pouch
PN, prenasal sac
SN, septalis
Sk, cranial wall
Sp, nasal septum
S, hypophysial branch of septalis
T, *n.* terminalis
T.&S, n. terminalis and n. septalis
TO, terminal organ
TH, hypophysial branch of terminal nerve
tt, terminal nerve bundle
V, velum
v, valve
Vt, ventricle

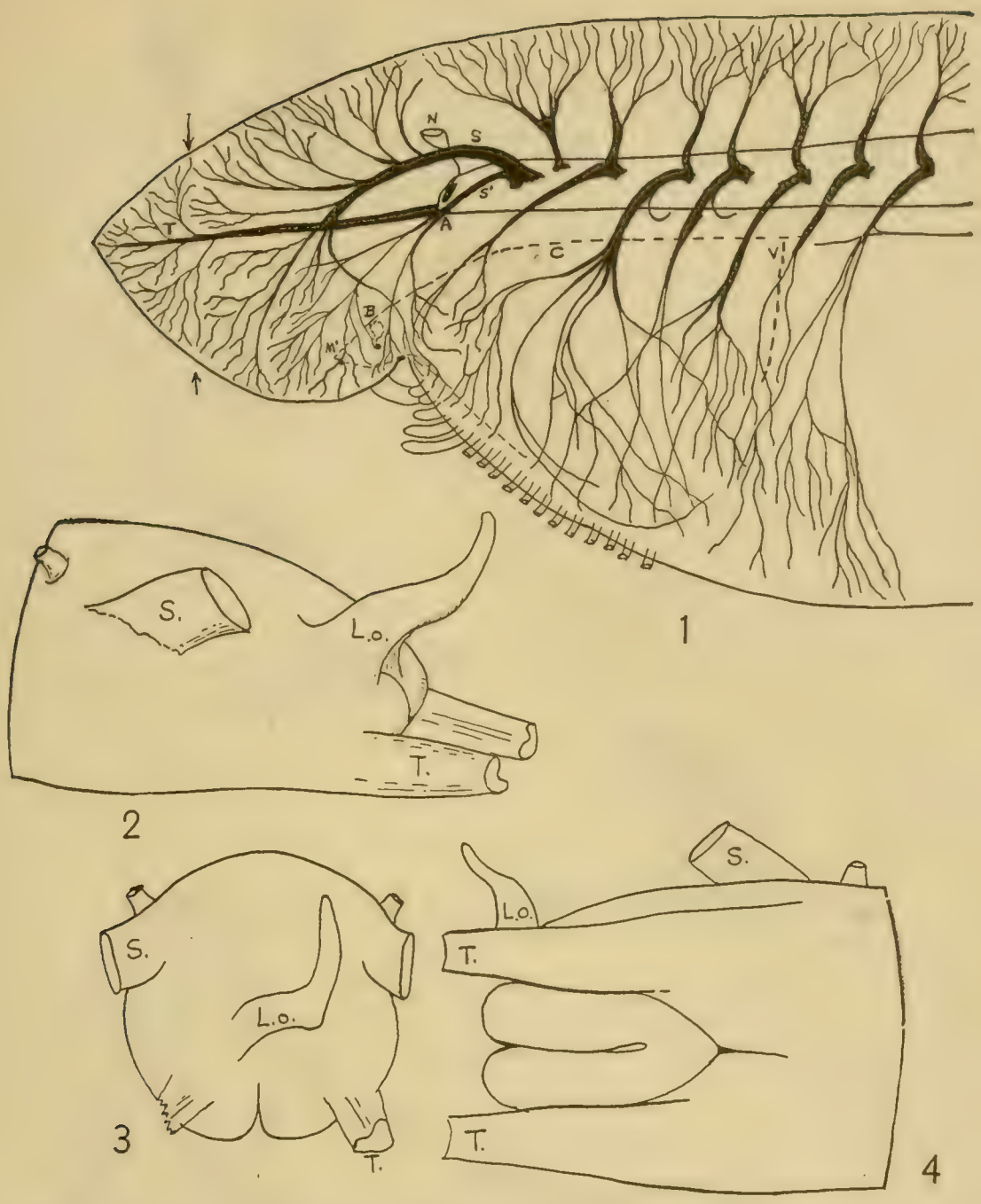


Fig. 1 View of left side of the head of Amphioxus, showing brain and nerves The terminalis, opticus, olfactorius, and septalis are the first four cranial nerves. The apical territory supplied by the terminal and septal nerves is distinctly marked off from the rest of the head and its close association with the optic and olfactory sensory structures is evident. The septal territory has already undergone partial migration into the buccal cavity. The path of migration of the hypophysial organ is indicated by the letters *A* and *B* and the dotted line *B–C*. Over the outline of the apical buccal pouch is shown the last surface position (in the larva) of the pre-oral pit (hypophysial organ) before its entrance into the mouth chamber.

Fig. 2 Anterolateral view of the brain of Amphioxus (short type), showing the terminal, septal, and olfactory nerves and the region of the lamina terminalis.

Fig. 3 Anterior view of the same brain of Amphioxus.

Fig. 4 Ventrolateral view of the same brain of Amphioxus, showing the first three nerves and the infundibular territory.

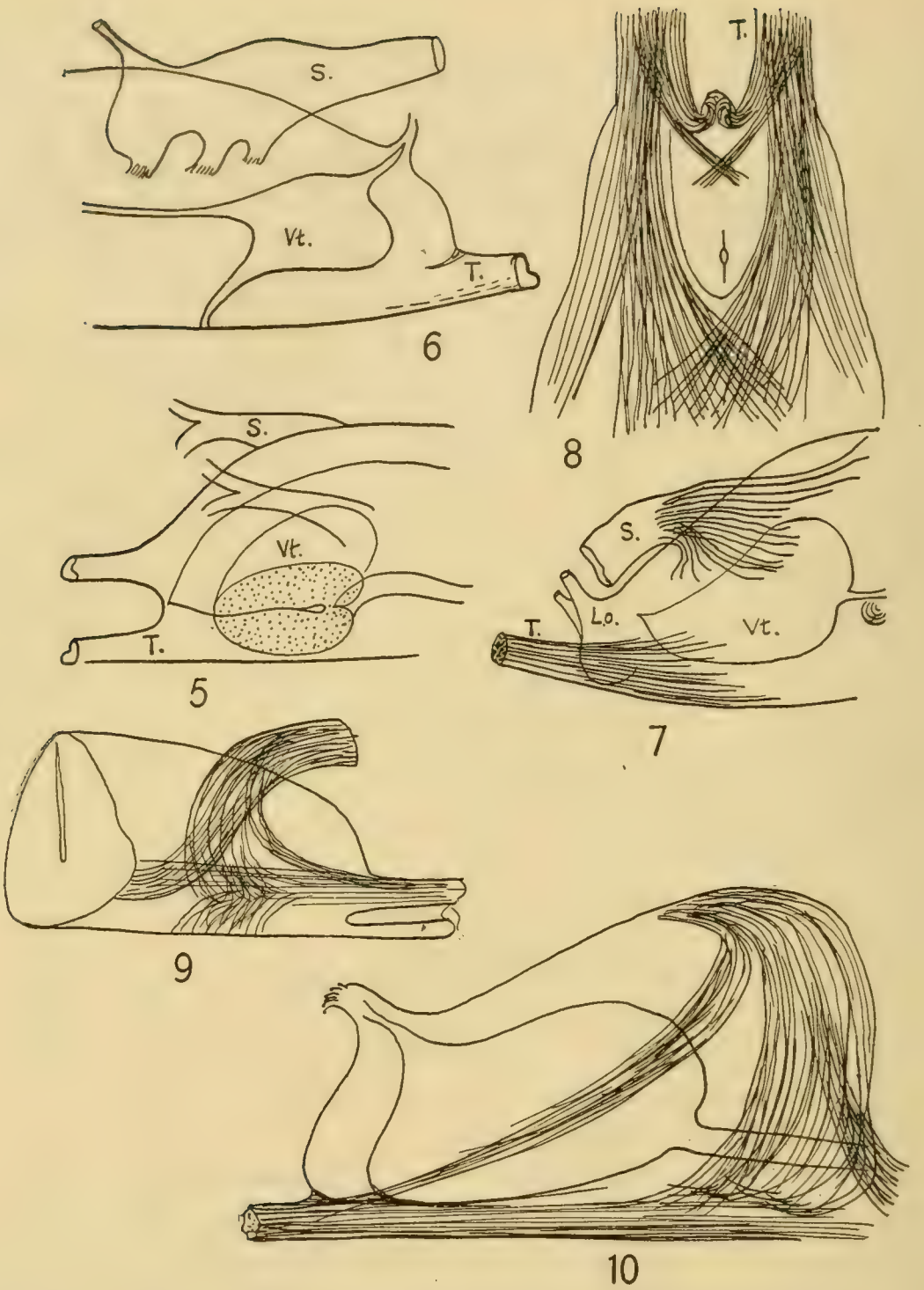


Fig. 5 Anterodorsolateral view of a brain of Amphioxus (transparent), to show the ventral territory of the neuropore in the adult.

Fig. 6 Lateral view of the brain of Amphioxus, to show the roots of the septal nerve, the ventricle with its olfactory, infundibular, and spinal prolongations.

Fig. 7 Dorsolateral view of brain of Amphioxus, to show paired olfactory nerves.

Fig. 8 Ventral view of brain of Amphioxus to show the course of fibers of terminal nerve.

Fig. 9 Posterior ventrolateral view of Amphioxus brain, to show course of fibers of terminal and septal nerves.

Fig. 10 Anterolateral view of another brain, to show course of fibers of terminal and septal nerves.

gether as a single nerve physiologically. The remarkable persistence of these two nerves as anatomically distinct structures throughout the entire vertebrate series from Amphioxus to man is noteworthy and needs further attention from anatomists. The concentration of the chemical sense organs, olfactive sense organs, and light perceptive organs at the anterior end of the neural axis is strikingly shown in Amphioxus and the anatomy of the nasal chamber of man as herein described shows that the morphological relations of these structures have not been much disturbed throughout the evolution of the vertebrates. In Amphioxus the neural lips of the anterior neuropore maintain their original relations in the adult. This is indicated by the position of the terminal and septal nerves which supply the tip of the head and the organs connected with the ventral end of the neuropore, the hypophysis, while the olfactory nerves supply the organs connected with the dorsal end of the neuropore, the nose. The whole neuroporic territory is concerned with testing alimentary and respiratory supplies, i.e., food in the broad sense.

2. AMMOCOETES AND PETROMYZON

The head region of Ammocoetes has the shape of a truncated cone with the snout truncated from above, downward, and backward. In the larval Petromyzon the superficial territory innervated by the terminalis and septalis which in Amphioxus forms part of the body surface is withdrawn into the protection of a nasal canal along with the olfactory organ and opens secondarily to the outside through the nasal canal. Confining our attention for the present to the morphological equivalents of the parts just described in Amphioxus, we find the spear-shaped primitive tip of the head withdrawn bodily into the nasal chamber to form the nasal septum of Ammocoetes, each half receiving a rich nerve supply from its N. terminalis (fig. 13). The characteristic condition of the septum in Ammocoetes furnished the key for the solution of the problem of the homologies of the nasal organ of vertebrates. On either side of this septum (fig. 14) we find the olfactive organs, each with its N. olfactorius. The N. septalis

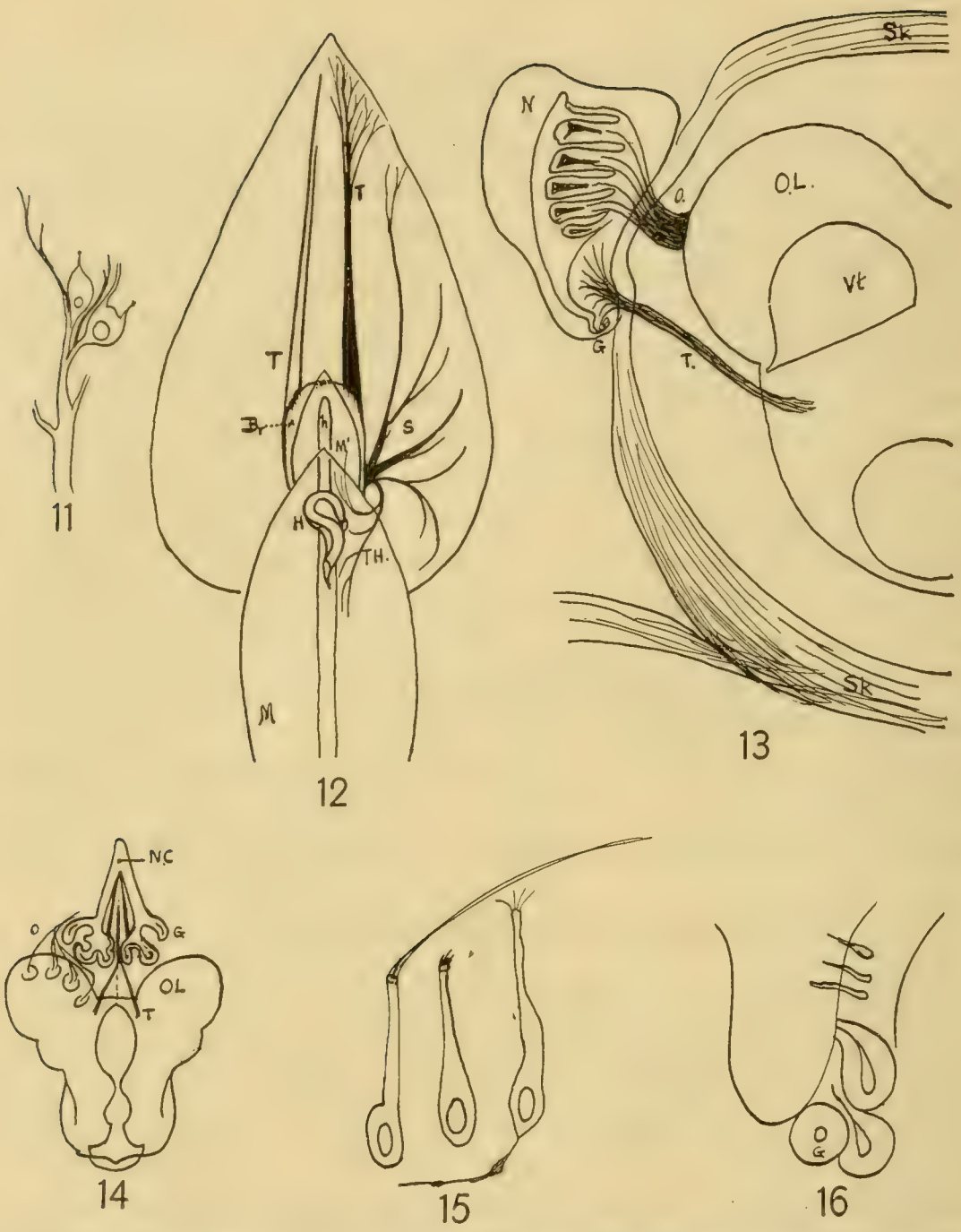


Fig. 11 End twig of terminal nerve with ganglion cells and peripheral fibrils

Fig. 12 Ventral view of snout and mouth of Amphioxus, to illustrate relations of brain to anterior buccal pouch, and the distribution of the terminal and septal nerves as seen from below. The hypophysial organ with dorsal buccal groove running caudad and the band of thickened epithelium running cephalad are shown. In the drawing the head is expanded laterally to make room to show the parts clearly.

Fig. 13 Sagittal section of brain and nasal organs of Ammocoetes, to show relations of olfactory and terminal nerves to the brain and the olfactory and terminal organs.

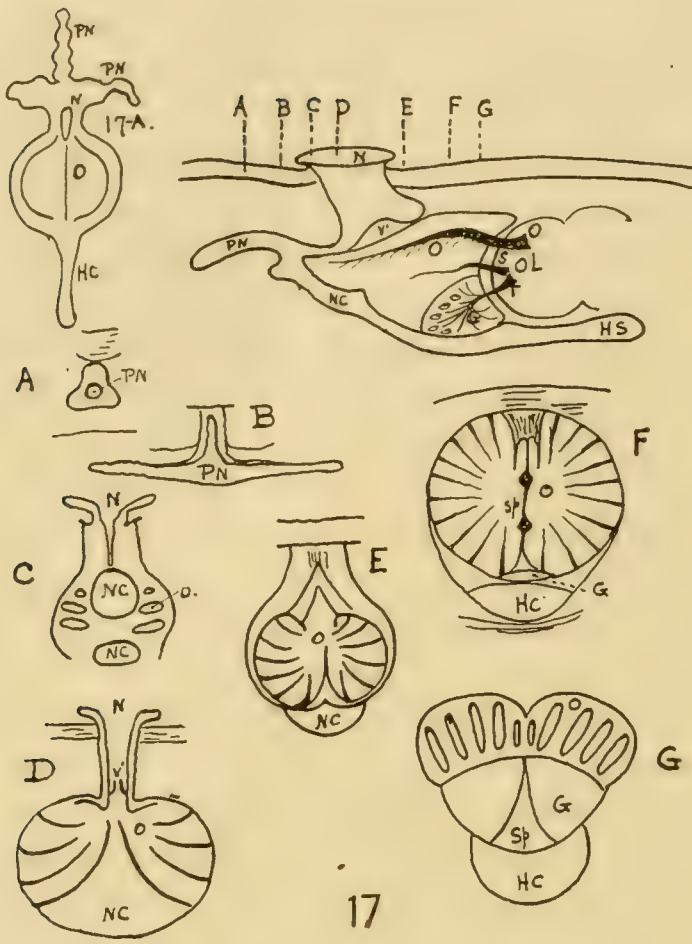
Fig. 14 Horizontal section of brain and nasal organs of Ammocoetes, to show relations of olfactory and terminal nerves and the nasal septum.

Fig. 15 Three cells from the 'nasal' epithelium of Ammocoetes, one olfactive sense cell and two ciliated epithelium cells from the ciliated 'gland' of the terminal organ.

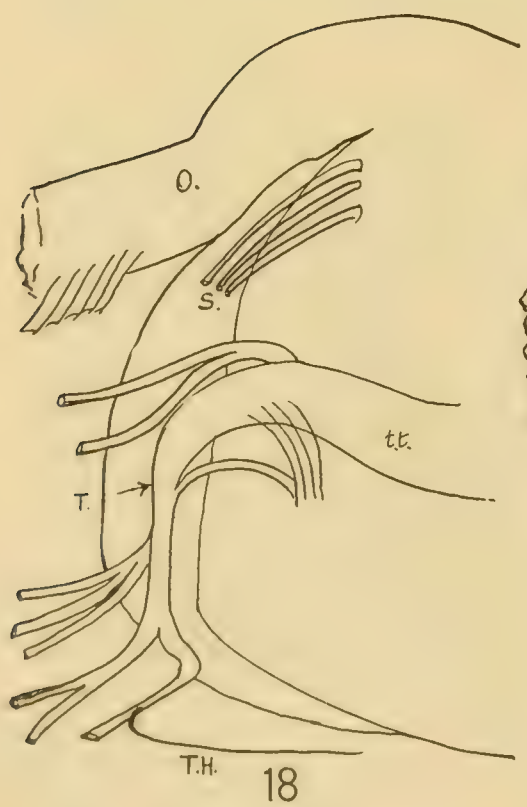
.Fig. 16 Part of a section of the 'gland' of the terminal organ.

passes to the septum along with the median bundles of the olfactory nerves. The optic nerves are well developed and leave the base of the brain below and behind the N. terminalis. Owing to the formation of heavy lips surrounding the mouth—which in the adult became the 'sucking disk'—the nasal tube opens on the top of the head in front of the brain region. The olfactory organs and nerves are completely separated from each other in Ammocoetes by this nasal septum, which from its origin we recognize as a fundamental as well as an original landmark in head anatomy. At the base of the septum is a glandular structure (figs. 14, 15, and 16) which is innervated by the terminal nerve. This paired gland is a more or less constant feature of the vertebrate nose from Ammocoetes up to man. It has been described in many forms as the organ of Jacobson. Between the Amphioxus and Ammocoetes condition of the terminal and nasal region of the head there has occurred a translation of this region ventrad and caudad in the sagittal plane with a concomitant enlargement of the nasal organ and the separation of its right and left halves by the interposition of a septum formed by the spear-shaped tip of the head. In other words, the olfactive organs have migrated ventrad along the sides of the septum. Thus the olfactive organs come to lie laterad of the terminal and septal territory, instead of dorsal, as in Amphioxus.

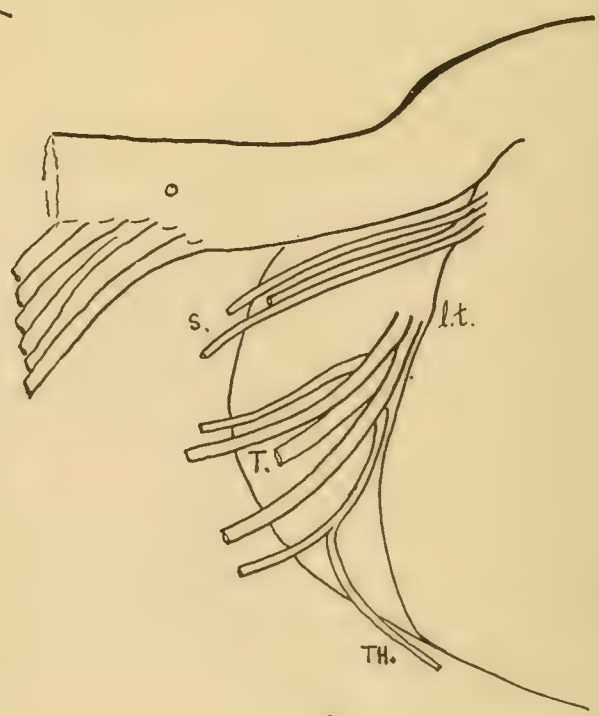
In Petromyzon marinus the tip of the primitive head has been sunk still further below the surface of the body and surrounding it the nasohypophysial canal has been complicated by the formation of sacs and pockets with valves and folds for the reception and control of the water to be tested. The olfactive organs have been expanded and pushed forward as well as downward, while the terminal and septal structures occupy the ventroposterior portion of the nasal capsule. This portion of the complicated nasal organ has been described as a gland. It is made up of a series of pockets or tubes which open out on the face of the wedge-shaped terminal organ to become continuous with the folds of the septal region of the nasal chamber (figs. 17 and 17A).


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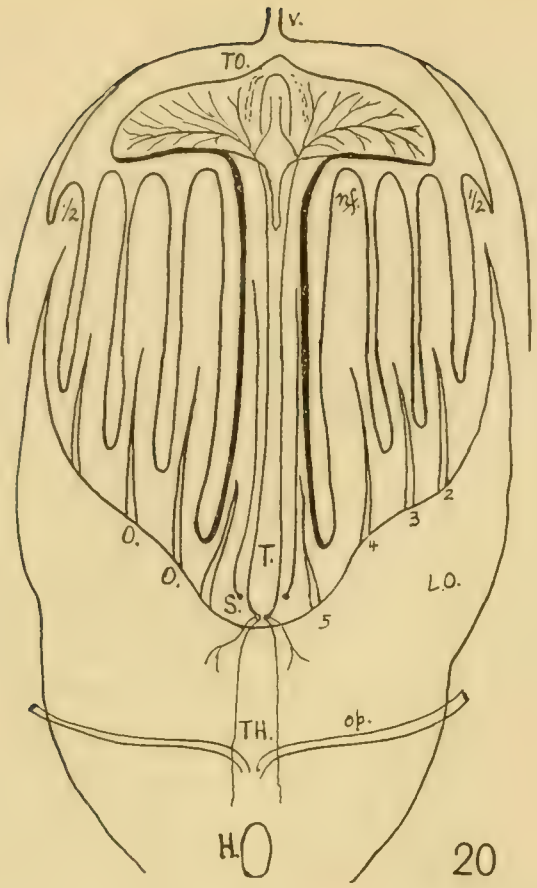
3. BDELLOSTOMA

In Bdellostoma the terminal region of the Amphioxus head has been withdrawn still deeper into the head of the adult hagfish by the formation of a long and capacious nasal canal (nasohypophysial canal) (figs. 21 and 22). The N. terminalis supplies a well-developed sense organ having to do with the testing of the respiratory water (fig. 20). About it are developed valvular folds from the lining of the nasal canal which control the admittance of the water to the terminal organ, the septal epithelium, and the nose and of course to the hypophysial canal. The nasal respiratory mechanism can quickly close off the sense-organ chamber and expel the water in the nasal canal forward as well as backward. The optic nerves are long and extend outward from the base of the brain to the optic cups, no lens being present. The N. terminalis runs dorsad within the brain (figs. 18 and 19) before breaking through to the surface and then runs ventrad within the median fissure between the olfactory lobes to the horizontal level of the terminal organ when it runs cephalad to its terminations in the tip and sides of this structure. The N. septalis leaves the dorsal region of the olfactory lobe of the

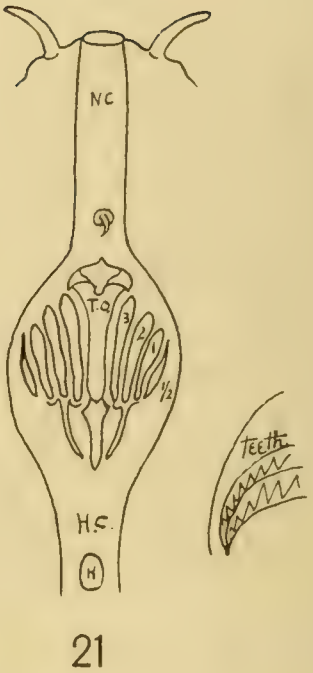
Fig. 17 Lateral view of the 'nasal' organ of Petromyzon marinus. The reference letters show position of sections A to G, figured below. 17A Is a dorsal view of the nasal organ of the same Petromyzon. A. Section through tip of prenasal sac. B. Section through expanded portion of prenasal sac. C. Section through the edge of nasal funnel, the nasal canal, the anterior ends of the anterior olfactory pockets, and the naso-hypophysial canal. D. section through the nasal funnel, showing the funnel valve and the anterior part of the nasal sac with the free folds. E. section through the posterior part of the nasal funnel, the body of the nasal sac with free nasal folds, the ventral half of the nasal septum and the nasohypophysial canal. F. section through the nasal sac with completed septum and free nasal folds in right and left nasal chambers, the anterior end of the nasal gland (terminal gland) and the nasohypophysial canal. G. section through the posterior part of nasal body showing posterior nasal pockets, the enlarged posterior end of nasal gland, the ventral part of septum and the nasohypophysial canal.

Fig. 18 Lateral view of the right olfactory, septal, and terminal nerves of Bdellostoma dombeyi, inside the median fissure separating the olfactory lobes. The dissection exposes the intercerebral course of the terminal nerve for a short distance.

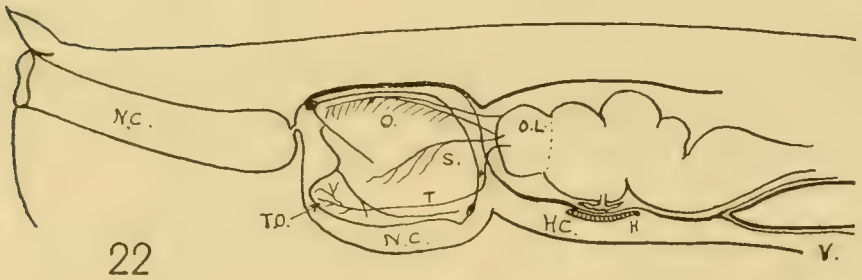
Fig. 19 Same view as figure 18 of another Bdellostoma brain.


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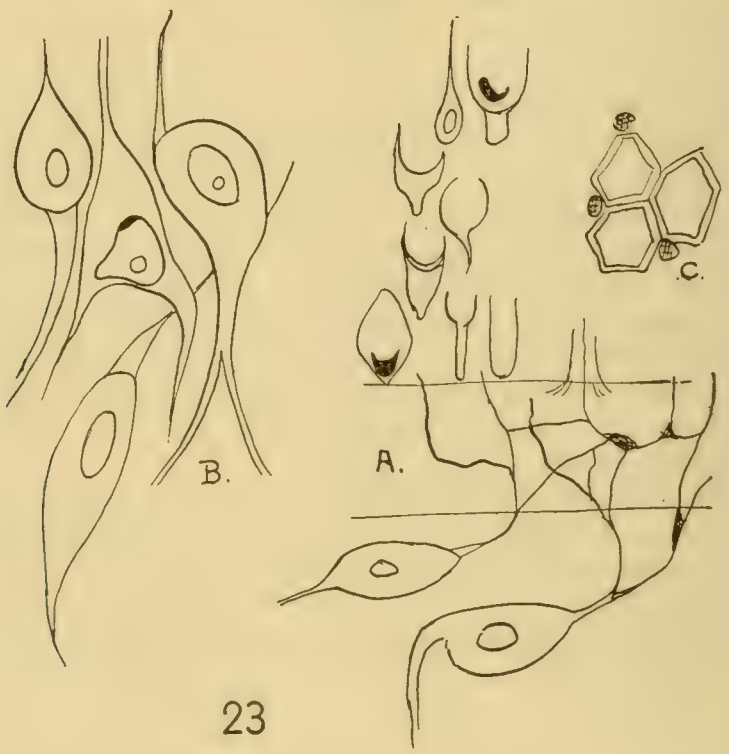
20


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21


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22


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23

brain inside the median fissure and near its anterior end, but separate from the median olfactory nerve bundle which leaves the tip of the median part of the olfactory lobes. The septal nerve also runs ventrad before coursing forward to supply its peripheral territory. The olfactory nerves are enormously developed and the nerve of each side supplies three complete and two half folds (plates) of the compound nasal organ, all of which, heretofore, has been called the olfactory organ. The N. terminalis in Bdellostoma presents a stage intermediate between Amphioxus and the Selachians.[3] In the former, the olfactory nerves are small and in their primitive terminal position on either side of the dorsal end of the neuroporic raphé, while the N. terminalis is relatively large, leaving the brain nearly midway between the olfactory organ and hypophysis, and strictly terminal in its positions as regards the adult brain.

In Bdellostoma the olfactory nerves have become enormously increased and overshadow the N. terminalis, which, while it remains terminal in its peripheral distribution leaves the brain near the anterior end of the olfactory lobes within the median fissure. The development of the olfactory lobes is so great that the primitive anterior end of the brain is covered over and its relation obscured. The great increase in size of the olfactory nerves causes them to enfold the forebrain in an inclosing overgrowth of the olfactory lobes, which apparently forces the roots of the terminalis upward, but they retain their relative position with reference to the exit of the olfactory nerve, viz., mesad and ventrad of it. In Amphioxus we found branches of the septal nerve

Fig. 20 Ventral view of Bdellostoma 'nasal' organ, to show the distribution of the olfactory, terminal, and septal nerves, the median terminal organ and other 'nasal' folds.

Fig. 21 Same view of nasal organ and nasohypophysial canal in Bdellostoma.

Fig. 22 Lateral view of nasal organ, brain, and nasohypophysial canal of Bdellostoma.

Fig. 23 A. Section through the mucosa covering terminal organ of Bdellostoma. Five layers of epithelium cells are shown above the basement membrane beneath which lies a plexus of nerve fibrils given off from the ganglion cells lying below. B. Four ganglion cells from same section. C. Surface view of epithelium from same terminal organ to show relation of sensory cells to other surface cells.

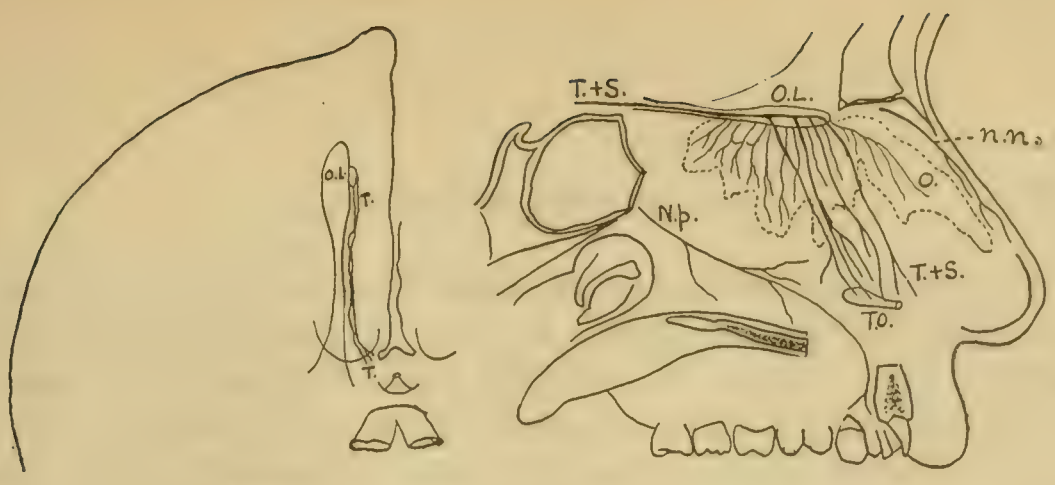
innervating the hypophysial organ. In Bdellostoma the hypophysial branches are given off from the terminal nerve. In mammals it has been shown by Huber and Guild[4] and Larsell[5] that both terminal and septal branches run to the organ of Jacobson, i.e., the terminal organ. In Bdellostoma, the ganglion cells of the terminal nerve lie in the terminal organ (fig. 23).

4. CHIMPANZEE AND MAN

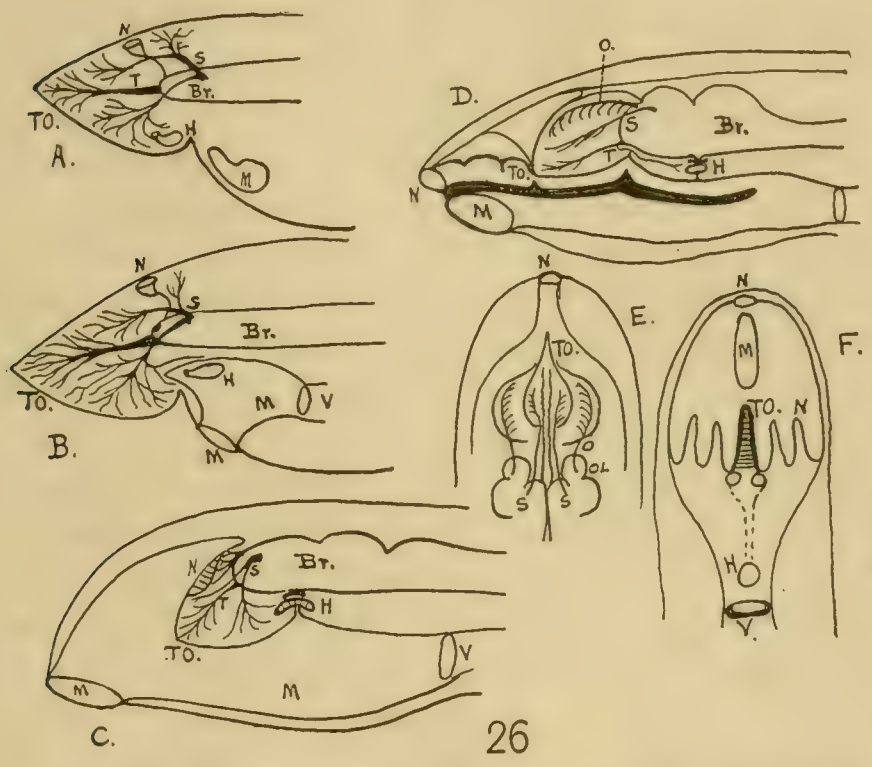
In man as described by Brookover[6] and in the chimpanzee, as my dissections disclose (fig. 24) the N. terminalis leaves the brain ventrad and mesad of the olfactorius and passes forward to the lamina cribrosa. In man they pass through this plate along with the septal and olfactory nerves and run ventrad to terminate in and about Jacobson's organ. In the chimpanzee the nerve on leaving the brain enters the pia and takes its course forward to near the lamina cribrosa where it passes into the dura and leaves the cranial chamber along with the bundles of olfactory fibers. Its course outside the cranial chamber was not traced. The recently published researches of Larsell[5] on several mammals show conclusively that both the terminal and septal nerves are, in this class, preserved in their original relations to the olfactory nerve and brain. The nasal chamber in man, therefore, contains the surface distribution of these three most ancient cranial nerves as well as the surface terminations of invading branches of a fourth and more recent cranial nerve, the trigeminus (fig. 25).

5. THE ANTERIOR CRANIAL NERVES

We thus find that the nasal septum and related parts form one of the most ancient and least changed morphological complexes in vertebrate anatomy. Reacting to physiological necessity, the advanced outposts of the central nervous apparatus of vertebrates were withdrawn early in the life of the phylum more or less deeply into the protective hood furnished by the overgrowth of the muscles supported by added skeletal structures, and body covering from territory lying behind the region of the primitive



24 25



26

Fig. 24 Ventral view of right cerebrum of Troglodytes (chimpanzee) to show intracranial course of N. terminalis.

Fig. 25 Sagittal section of nasal chamber of man from Toldt, to indicate extracranial course of N. terminalis drawn in from Brookover's description. The septal nerve (Vomeronasal) practically parallels the course of the terminal branches. The invading branches of the trigeminus are also shown.

Fig. 26 Six diagrams illustrative of the translation of the chemical sense organs of the neuroporic territory of Amphioxus, from the exposed position on the surface of the head to the inclosed condition found in Bdellostoma where they are housed in the 'nasal' chamber. A. Pre-Amphioxus stage with the hypophysial organ still outside of and in front of the buccal cavity. B. Amphioxus stage with the hypophysial organ inside the buccal cavity, said organ being the first of the apical territory to find protection. C. Post-Amphioxus stage in which the overgrowth of the trigeminal structures has housed in the remainder of the apical territory of the Amphioxus head. This apical region furnishes the nasal septum together with the sensory structures associated with it in the nose of the higher vertebrates. D. Bdellostoma stage in which a nasopharyngeal partition has appeared partly separating nasal and buccal chambers. E. Ventral view of stage C. F. Ventral view of stage D.

brain, in fact, from the territory of the trigeminus nerve; we therefore find that the advanced outposts of the brain, the most anterior sense organs of the body surface, in all forms above Amphioxus are tactile sense organs of simple or complex structure belonging to the trigeminus as distinguished from the earlier group of chemical sense organs, belonging to the terminal, olfactory, and septal nerves which form the anterior sensory outposts in Amphioxus. This change in the character of the sensory outposts is of course a result of forward overgrowth of the trigeminus mechanisms. We also find that the trigeminus structure has not only surrounded and housed in this group of chemical sense organs, but has also invaded the original olfactory and terminal sensory territories supplied by the NN. terminalis, olfactorius, and septalis, and here performs some function of a tactile sort. Along with the housing of the chemical sense organs there has been built up in the long series of vertebrate forms a large variety of control systems of valves, doors, guide folds for the control of water and air currents, their admission, guidance, and expulsion varying in different animals as the case may be. The emergence of vertebrates from water breathing to air breathing has not affected the physical conditions of the functioning of the chemical sense organs, for they still are kept wet and pick up their stimuli out of a liquid medium, thanks to the moisture supplied by the 'mucosa.' With the exception of the jaw apparatus and related parts, no single change of, or addition to head structures has caused greater changes in contour or anatomical detail than the housing of the chemical sense organs. Owing to the failure to recognize the presence of both the terminal and septal nerves, the first arising ventrad of the olfactorius, while the second arises dorsad of the olfactorius, much confusion exists in accounts of the so-called nervus terminalis. In all vertebrates yet examined, one of these nerves is found to be present, which may arise near the dorsal surface of the brain or near the ventral surface. In many forms both pairs of nerves have been found. Further investigation will doubtless show that both nerves are present in all vertebrates. In order to make final decision, it will be necessary to trace the

nerves to their central as well as peripheral terminations. The presence of coördinating sympathetic fibers in the terminal and septal nerves seems to be definitely proved in a number of the species studied. The diagrams shown in figure 26 cover four stages in the transformation of the apical head region of Amphioxus into the nasal chamber and septum of the higher forms.

Stage A is pre-Amphioxus condition. The hypophysial organ (pre-oral pit) is shown on the outside of the head in the position it reaches during larval life just before it enters the buccal chamber. Here it is in the surface territory innervated by the septal nerve.

Stage B represents the relation of the parts in the adult Amphioxus. The hypophysial organ is housed in the buccal cavity.

Stage C is a pre-Bdellostoma condition antedating the formation of a nasobuccal partition. The entire head territory of the Amphioxus stage is now housed in the buccal chamber and projects into it along the sagittal plane, forming a partial septum which partly separates the nasal portion of the common nasobuccal chamber into right and left nasal chambers.

Stage D represents the Bdellostoma condition. The primitive apical territory is now enclosed in a 'nasal capsule,' open below, which is divided into right and left halves by the terminal organ, which forms a complete septum for structures of the capsule. The nasal chamber is further separated from the buccal cavity by a horizontal partition. The nasohypophysial canal is an extension forward and backward of the nasal chamber.

The cranial nerves in man are not, therefore, twelve in number, but fourteen. In the order of their connection with the brain we may tabulate them as follows:

Nerve 1, terminalis	Nerve 8, abducens
Nerve 2, opticus	Nerve 9, facialis
Nerve 3, olfactorius	Nerve 10, acusticus
Nerve 4, septalis	Nerve 11, glossopharyngeus
Nerve 5, oculomotorius	Nerve 12, vagus
Nerve 6, trochlearis	Nerve 13, accessorius
Nerve 7, trigeminus	Nerve 14, hypoglossus

6. COMMENTS ON FUNCTION

We know almost nothing of the function of the terminal organ, of Jacobson's organ, of the hypophysis. We are perhaps warranted in assuming that the terminal, olfactory, and septal nerves have to do with special chemical senses, of which above Amphioxus the olfactory sense plays the predominant rôle. Next in importance stands the terminal sense organ, which we find from the fishes on, as the organ of Jacobson, a paired sense organ of the nasal chamber which appears reduced in importance as compared with the olfactory organ, although in the Ophidia the reverse appears to be the case. The functions of the nerves in the nasal chamber may be divided as follows:

Chemical sense.....	Olfactory nerve	Testing alimentary foods
	Terminal nerve	Testing respiratory foods
	Septal nerve	
Tactile sense.....	Trigeminal nerve	Testing for solid bodies in the respiratory currents and sensing the pressure and the current flow

Although the chemical sense organs have been housed in the nasal chamber, they have, so far as the structure of the sensory surfaces are concerned, remained in a primitive state. These sense organs have not developed accessory parts in such degree as have the eye and ear. In the case of the eye, the vitreous body, lens, cornea, lids, etc., have been added to increase the precision or enlarge the range of its functions, there has been added to its light-perceiving function the optical reactions. In the case of the ear, there has been a parallel evolution of the primitive function of wave-motion perception by the addition of tone perception, with the cochlea as its anatomical expression. In both cases there has been a progressive increase in the number of protective structures as well as of parts serving the increase of precision and enlarged range of function. In the case of the chemical sense organs of the nasal chamber there has not been such considerable increase of subsidiary parts or perfection of the sensory structures, and they therefore remain organs whose stim-

uli belong more to the subconscious domain of reaction to the environment than is the case with either the eye or ear. Even when conscious attention is directed to the reactions of the nasal organs, they can only partly be brought into the realm of definiteness. This is proved by the fact that from the days of Aristotle down to the discovery of the terminal nerve there was never a hint of anything more than an olfactive function. Even in man to-day the olfactive function is a vague and uncertain sense in itself and needs, in order to make certain the interpretation of the stimulus, the assistance of other sense organs, e.g., the eye or the ear. To illustrate, most persons are sure they can recognize the odor of the rose, specifically, a given variety of rose, with whose characteristics they are familiar, but blindfolded and lacking tactile stimuli they cannot identify with certainty the source of the odor, often indeed it may call up the memory of violet odor or some other odor. It is different with the eye. By sight alone and within a considerable range of distances we can recognize any object of definite form which we have seen before. The ear stands between the nose and eye with regard to definiteness and certainty of the results of the stimuli. While we can 'smell' an odor and not be able to identify it, and can hear a tone and not be able to place it in the scale, we can always recognize objects by sight, by either their form, color, size, motion, or all combined. All three senses are quite equally and similarly limited by the upper and lower limits of intensity of stimuli. Although the nasal senses lack conscious definiteness, when compared with the eye or ear, they are not on that account less determinative of physiological (and psychological) reactions. They are primitive and fundamental senses. When stimulated, the nerve reactions, even though subconscious, may be propagated far and wide throughout the nervous apparatus, much like 'sympathetic' reactions. Rarely is there an instantaneous response such as so frequently results from stimuli of the auditory nerve. The cranial nerves of the nasal chamber have not the intimate associations with the 'voluntary' muscular apparatus that the auditory apparatus has. Our knowledge of both the peripheral and central rela-

tions of the four cranial nerves having endings in the nasal chamber is very imperfect. Even more fragmentary and obscure is our knowledge of their functions.

Winding Way and Valley Road
Cincinnati, January 20, 1919

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Resumido por el autor, Leslie B. Arey.

Un mecanismo retinal para la visión eficiente.

Las células visuales y el pigmento retinal de muchos vertebrados inferiores exhiben sorprendentes movimientos a la luz y en la oscuridad. Los experimentos comunicados previamente han establecido que la rapidez de estos cambios ha sido muy exagerada. La validez de la suposición, también muy extendida, respecto a su umbral de sensibilidad extremadamente bajo, ha sido investigada después por el autor. Tal determinación es importante en vista de otra suposición discordante que considera que los cambios en la posición de las células visuales a la luz intensa y a la difusa favorecen la visión de los conos y bastones, respectivamente, mientras que los movimientos correspondientes del pigmento retinal también aumentan mecánicamente la eficiencia visual. En otras palabras, si los beneficios reputados como adaptativos se derivan de tales cambios fotomecánicos, las reacciones a la luz difusa deben ser esencialmente idénticas con las que se sabe ocurren en la oscuridad total. Esta suposición sin embargo, es en su mayor parte gratuita. Las reacciones de estos elementos bajo la acción de la luz de intensidad graduada prueban que el umbral de estimulación es notablemente elevado. En general, el máximo de reacción hacia la luz aparece primero en una intensidad luminosa que permite justamente la lectura de los caracteres de imprenta ordinarios. De aquí que la supuesta sensibilidad fótica elevada de las células visuales y pigmento retinal no queda probada, mientras que las condiciones mecánicas para una visión de la penumbra, teóricamente más eficiente, se establecen sobre una base experimental.

Translation by José F. Nonidez
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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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A RETINAL MECHANISM OF EFFICIENT VISION[1]

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TWO TEXT FIGURES

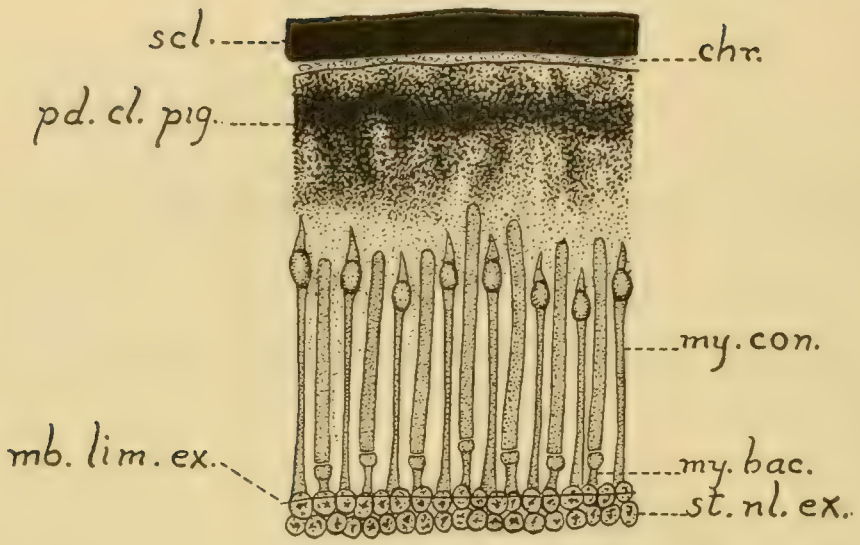
PRELIMINARY

It is a well-established fact of retinal physiology that the visual cells and retinal pigment in many of the lower vertebrates exhibit striking movements in light and in darkness ('15 a, '16), although in man and other mammals such changes apparently are slight ('15 b).

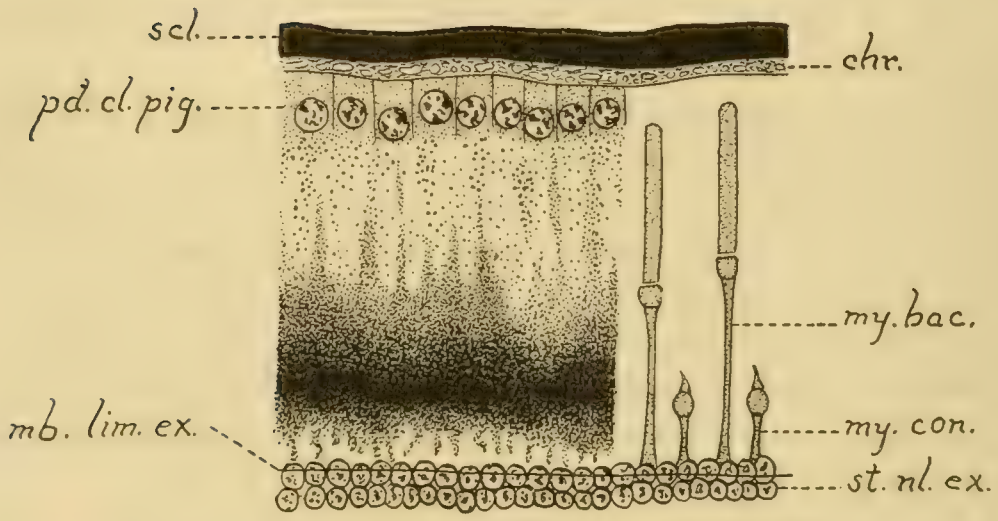
The pigment cells have processes, probably fixed, which interdigitate with the visual elements and in which the pigment granules migrate to and fro (figs. 1 and 2). The visual cells likewise modify their positions due to the contractility of the so-called myoid, which is that portion of the inner member of the rod or cone between the ellipsoid and the external limiting membrane. The extensibility of the myoid is variously developed, but in some animals the extremes of change may be as one is to ten ('16).

There is represented in figures 1 and 2 the condition of the retina, with respect to these changes, as it is characteristically found in a fish, the common horned pout, Ameiurus. In darkness (fig. 1) the pigment withdraws toward the chorioid, thus exposing the visual rods and cones; of the two types of visual elements the cone is extended, whereas the rod is so retracted that its ellipsoid lies close to the external limiting membrane. In light (fig. 2) the appearance is reversed; the pigment now

[1] Contribution No. 69, March 3, 1919. The experimental data were obtained at the Fairport Biological Station while a guest of the United States Bureau of Fisheries.



1



2

Fig. 1 The effect of total darkness on the position of the retinal pigment and of the visual rods and cones of the fish Ameiurus nebulosus. The pigment is withdrawn toward the chorioid; the cone myoid elongates, the rod myoid shortens.

Fig. 2 The effect of bright, diffuse daylight on the same retinal elements of Ameiurus. The pigment moves forward toward the external limiting membrane, thereby masking the visual rods and cones. At the right of the figure the positions of these visual cells are indicated—rods elongated, cones shortened.

ABBREVIATIONS

chr., chorioid; *mb.lim.ex.*, external limiting membrane; *my.bac.*, rod myoid; *my.con.*, cone myoid; *pd.cl.pig.*, base of pigment cell; *scl.*, sclera; *st.nl.ex.*, external nuclear layer; *st.pig.*, pigment layer.

pushes outward to mask the visual cells, while these latter occupy mutually reciprocal positions—rods elongated, cones shortened.

Such positional changes have been interpreted as of use in furthering efficient vision. It seems logical that the masking pigment of the light phase would serve to protect the delicate visual elements from the overstrong influence of light (Chiarini, '06), while the insinuation of such pigmented processes between the individual visual elements effects for the latter a certain degree of optical isolation by acting as an absorbent of dispersed rays refracted from neighboring cells (Garten, '07). From the standpoint of sensory reception, the sharpness of the retinal image is in this way enhanced. The withdrawal of the pigment in dim light might be thought to involve a response which allows the visual cells to utilize all the weak light available.

Furthermore, there is good reason to believe that the cones are concerned with bright-light vision, the rods with dim-light or twilight vision. Hence a shortening of the cone in bright light, drawing it down nearer the source of illumination, while the rod at the same time elongates and is thus moved out of the way, would appear to be a useful maneuver (Herzog, '05; Exner and Januschke, '06). The converse procedure in dim light—by which the rods are shortened and the highly refractile cones, with their lens-like ellipsoids no longer masked by pigment, are lengthened—would be equally advantageous (Garten, '07; Arey, '15 a).

For the detailed applications of these apparently adaptive responses the reader is referred to Garten ('07), who gives an extended consideration of the correlations within the vertebrate classes between the morphology, optical qualities, and distributional ratios of the rods and cones on the one hand, and, on the other, their movements together with the migrations of the retinal pigment.

Interesting and logical as these speculations may be, they nevertheless lack a sound experimental basis. It is certain that the movements as summarized in a preceding paragraph occur respectively in daylight and in darkness; but since responses in total darkness cannot be useful in the manner suggested, it is obvious that in order to derive the reputed adaptive benefits

from such photomechanical changes, the responses in dim light must be identical with those demonstrably occurring in the dark. This assumption, however, is largely gratuitous.

The supposition of an identity between the set of responses ensuing in dim light and in darkness is further complicated by certain conflicting statements and beliefs. There is a general impression current that the retinal pigment and visual cells react to the slightest traces of light; this is reflected in the statements of many workers who have feared their results would be impaired unless the strictest precautions were observed.

On the basis of actual experimentation, however, the earliest observations are encountered in the writings of Angelucci ('90), who reported that five minutes of candle light caused the frog's cones to be highly shortened, whereas the pigment remained as in darkness. On another page, nevertheless, he records that after twenty minutes of twilight the pigment assumed the light position, but the cones are influenced to a less degree!

Somewhat later, Pergens ('97) wrote that after a five hours' exposure to colored lights (red, yellow, green, and blue) of an intensity such that colors could be distinguished by an observer after one minute of dark adaptation, the cones of the white fish, Leuciscus rutilis, are strongly retracted. The weakest response was reported from the blue—a result, however, not in agreement with Herzog's ('05) later findings on the frog. The latter worker found the blue-violet most effective, although it should be added that the duration of exposure employed by him was only two minutes.

In a further communication ('99) Pergens confirmed his earlier conclusion that the pigment migrates least extensively in red light (equal intensities being used), but mod fied his previous belief regarding the inefficiency of the blue to provoke cone retraction.

Exner and Januschke ('05) performed some experiments, which, unfortunately, are not trustworthy as evidence. Specimens of the fish Abramis brama were exposed during the late afternoon, the experiment continuing through the period of failing light and terminating at dusk. Examination showed the cones to be in the position characteristic of light.

In a few experiments Garten and Weiss ('07) found that light in which colors could not be recognized, acting for five or more hours, caused the cones of the frog to assume a position intermediate between that characteristic of bright light and of total darkness. A limited pigment migration was reported as well.

These same workers made observations on the fish Abramis brama, contained in a dish lighted by reflection from the cover. Two grades of illumination were chosen: in one colors held within the container could not be distinguished; in the other they were recognizable. According to the results given, in the first grade the cones were maximally retracted in nine retinas and partially so in seven. In the second grade the cones were found shortened in all the eight retinas used, whereas the pigment exhibited no noteworthy change except in the sector constituting the ventral one-third of the eye.

The foregoing statements reveal the following conditions: Angelucci's several pronouncements in the same publication, if not actually contradictory, at least serve to befog the issue. The use of colored lights by Pergens was unfortunate; moreover, the reliability of certain of his conclusions is questionable. Exner and Januschke's experiments were so ill devised as to furnish no crucial evidence. The results of Garten and Weiss suggest an extreme sensitivity of the cones to low light intensities, whereas the pigment patently has a higher threshold. Finally, there exist no data concerning the responses of the rods to weak light.

It is clear that if the visual elements and retinal pigment are as highly sensitive to mere traces of light, as often has been held, they can assume no useful positions in the ordinary dim light of rod vision, while the utility of a response evoked under conditions of virtual darkness will still await an explanation. Accordingly, it was with the intent of learning the true conditions that this investigation was undertaken.

PROCEDURE

Essential to success in a determination of this kind is the choice of appropriate experimental animals. Previous experience with a variety of forms led to the selection of two fishes and the frog. The cones of the golden shiner, Abramis crysoleucas, have large, conspicuous ellipsoids and are so highly mobile that the light-adapted myoid can shorten to one-tenth its maximum length in darkness ('16). The cones of the common grass frog, Rana pipiens, are easy to observe, but show a narrower range of movement, the limits of extensibility being as one is to four. The rods of the horned pout, Ameiurus nebulosus, not only are of exceptional size in comparison with the usual diminutive elements of fishes, but they also undergo changes in length in the ratio of one to ten ('16); the value of Ameiurus for experimentation of this sort cannot be overestimated. There is a further inherent advantage in the animals chosen, inasmuch as their visual cells remain at a uniform level during the characteristic positional changes. The retinal pigment of all three animals exhibits extensive movements: in darkness it is confined to a narrow zone at the bases of the pigment cells, whereas in the light it migrates nearly to the external limiting membrane.

Temperature is an additional factor which must be carefully controlled, although the necessity of this has been recognized only recently ('16). In dark-adapted fishes a temperature near the freezing point brings about a maximal contraction of the cone myoid, such as is characteristically associated with the action of light, while raising the temperature to the limit which is compatible with life proportionately elongates the myoid. Light, however, is so much more effective than temperature that the latter factor does not enter as a complication in the light adaptation of cones. The relatively slight quantitative effects of temperature acting in darkness on the position of the rods and on the distribution of pigment may also be disregarded. In the frog, on the contrary, high and low temperatures evoke a maximal expansion from the pigment during dark adaptation, complete contraction being obtainable only at an intermediate grade of about 15°C.; the cones, however, are shortened at the

upper temperature limits alone. From these statements it follows that to obtain significant results from experimentation upon frogs a temperature of about 15°C. must be maintained, while with fishes ordinary summer temperature of 22°C., or higher, is favorable.

Experiments were conducted in a large, windowless room into which weak daylight of a non-directive nature could be introduced from a second room; the latter, in turn, received its light directly from windows located at the end far from the single communicating door. Animals were tested under five[2] different conditions of illumination: 1) total darkness; 2) light in which the presence of objects could just be determined; 3) light of an intensity which allows the certain identification of bright colors; 4) light which just permits the reading of ordinary journal print; 5) bright, diffuse daylight. Exposures lasted two or three hours or more.

As experience proved, these seemingly rough criteria of light intensity are sufficiently accurate for the purposes required; with a little practice such grades can be kept fairly uniform. Permanent preparations of Perenyi-fixed, paraffin-infiltrated sections served as a basis for study.

To further brevity and clearness, the results obtained from experimentation will be condensed to mere summaries.

EXPERIMENTATION

A. Retinal pigment

1. Frog. In total darkness, and in light of just sufficient strength to allow objects or colors to be discerned, the pigment lies in a narrow stratum near the chorioid. When the illumination is increased just sufficiently to allow the reading of ordinary print, the pigment, for the most part, becomes expanded, in some cases completely, in others in a zone only three-fourths the maximal breadth.

[2] For Ameiurus another intensity—one which enables ordinary print to be easily read—was also utilized.

2. Ameiurus. The pigment of this fish is at first more sensitive than that of the other animals studied, migration being distinctly initiated in many (or perhaps most) individuals at an intensity by which the presence of objects can be detected. At the next grade (colors distinguished), expansion is well advanced, although the pigment does not extend the maximal distance, for it is dense at the cell bases, but sparse distad. In light in which one can just read, the expansion is nearly complete, but does not become maximal until the illumination is sufficient to allow easy reading.

3. Abramis. The effect of light is not apparent until it is of such a strength that colors can be recognized. At this intensity the pigment in about half the individuals was essentially in a condition of greatest contraction; the remainder showed the pigment well started, but not extended at most more than half the way to the external limiting membrane. Owing to a sudden failure in my supply of animals at the end of the season, I secured but few observations on the effect of light which makes reading possible; the pigment in those animals studied, however, was in a state of partial expansion only, so that it appears safe to conclude that stronger illumination is necessary to allow expansion to proceed to completion.

B. Visual cells

1. Cones. The cones of Abramis and the frog remain fully elongated, in the characteristic dark positions, until the illumination is so increased that colors are recognized. At this intensity the cones of Abramis show distinct indications of incipient retraction, while those of the frog are less than half their former length. When illuminated sufficiently to allow reading, the frog's cones shorten maximally; the cones of the few Abramis studied[3] were likewise greatly shortened, but not completely so.

[3] As on the tests on the retinal pigment of this animal, further experimentation would have been desirable, but the supply of available material suddenly ceased.

2. Rods. Until an intensity is used at which printed matter can be read, the rods of Ameiurus remain in the typical shortened condition of darkness; at about this grade, however, they apparently begin to elongate slightly. Any considerable degree of lengthening must first appear only in stronger light.

DISCUSSION AND CONCLUSION

These results, as compared with the findings of Garten and Weiss (compare p. 347), indicate that there exists a rather lower degree of photic sensitivity in the visual cells and retinal pigment than they maintain. Nevertheless, it must be apparent to all, not only that in the subjective choice of arbitrary grades of light a variable personal factor enters, but also that the description of stages thus chosen cannot be expressed in accurate terms. It is possible, of course, for any one person to standardize these grades fairly well for his own experiments; on the other hand, the determination of a critical intensity, such as that in which 'colors can be distinguished,' depends on one's individual acuity in color discrimination, on the brightness of the test colors, and on the decision as to whether these colors are to be just distinguishable with intense scrutiny or to be identified with ease. In any event, it appears that my first grade of illumination (that in which objects were discernible) was undoubtedly of lower intensity than the weakest employed by Garten and Weiss (compare p. 347); it lay far below the point where colors cease to be recognizable, this latter constituting their lowest grade.

Moreover, it is not impossible that the intensity of light to which their animals were actually subjected was higher than Garten and Weiss supposed. Their animals, contained in a 'spacious basin,' received light (electric) reflected downward from the cover. After the experimenter had accustomed his eye to total darkness for five minutes, the condition of illumination was judged by looking downward into the dish at colors placed directly over the surface of the water. As to whether this is a method which tends toward underestimating the light

conditions actually obtaining in the basin, the reader may decide for himself.

There is one further circumstance which on casual consideration might be held responsible for the quantitative divergence between my results and those of Garten and Weiss. They continued their experiments in most cases for five hours, while my determinations lasted on the average perhaps three hours. It seems plausible that the long-continued action of very weak light might register an effect not manifest in shorter periods. That this possibility is not operative in the cases under consideration follows from certain other observations of Garten and Weiss. They report maximal cone retraction in the fish after the weakest grade of light employed had acted in one series for three hours and in another crucial series for one and one-half hours. Garten also records that in light too weak to distinguish colors by, a shortening of the cones occurred ('eintritt') in one hour.

The facts developed in this investigation may for convenience be consolidated into the following statement: although responses of the visual cells and retinal pigment may be initiated at lower intensities of light, an approach to a maximal response is first elicited at an intensity which permits the reading of ordinary print. *This signifies that the threshold of stimulation of the visual cells and retinal pigment is high; or, in other words, the assumed great photic sensitivity of these elements is disproved.* Furthermore, since the responses in weak light are substantially identical with those in darkness, the mechanical conditions are present for a theoretically more efficient dim-light and bright-light vision, as postulated (compare p. 345).

SUMMARY

The threshold of stimulation of the visual rods and cones and of the retinal pigment, at which they exhibit their characteristic photomechanical changes, is high.

The alleged great sensitivity of these elements to light of extremely low intensity is consequently disproved.

Although responses may be initiated at lower intensities, in general an approach to a maximal light response is first elicited at an intensity which makes ordinary print legible.

Since the responses in dim light approximate those in total darkness, the mechanical conditions are present for a theoretically more efficient dim-light and bright-light vision than would otherwise obtain. This increased efficiency depends upon the assumption by these elements of correlative advantageous positions.

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Resumido por el autor, Swale Vincent.

Contribución al estudio de los reflejos vasomotores.

La estimulación de los nervios sensoriales en perros anestesiados con éter o cloroformo produce generalmente un aumento en los movimientos respiratorios, cuando la narcosis no es muy fuerte o cuando no se emplea el curare. Este aumento de movimientos respiratorios produce una disminución de la presión sanguinea y cuando son pronunciados no se puede obtener ningún reflejo presor, aun estimulando fuertemente. Con narcosis fuerte o compresión del cerebro no se produce la disminución de la presión sanguinea debida a esta causa. La interferencia mecánica con la circulación es la causa de esta disminución, puesto que se elimina abriendo el tórax. Este fenómeno complica seriamente los experimentos sobre los reflejos vasomotores. En los perros anestesiados con éter o cloroformo o con el cerebro comprimido, una débil estimulación de los nervios produce generalmente una disminución, y una fuerte estimulación un aumento en la presión sanguinea. La frecuencia de la estimulación ejerce efectos sobre el reflejo; cuandó es rápida se obtiene un aumento, y con menor rapidez una disminución. Los nervios más voluminosos responden de un modo más marcado al estímulo que los menores. La estimulación de la piel, músculos e intestino origina generalmente una disminución en la presión sanguinea, pero si se estimula la piel de un modo violento y extenso se produce un aumento en dicha presión. Bajo la acción de la morfina y el curare, por el contrario, un aumento en la presión tiene lugar generalmente, aunque con la morfina la estimulación débil puede producir una disminución de la misma. La influencia de las glándulas endocrinas sobre los reflejos vasomotores no es clara (veáse sin embargo Pearlman and Vincent "Endocrinology," en prensa). El cambio de reflejo en la estimulación de los nervios somáticos se produce principalmente por los efectos sobre los vasos sanguíneos del área esplácnica.

Translation by José F. Nonidez
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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JUNE 2

A CONTRIBUTION TO THE STUDY OF VASOMOTOR REFLEXES

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NINETEEN FIGURES

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I. INTRODUCTION

General blood-pressure is affected reflexly by central stimulation of various sensory nerves (reflex vasomotor action). This subject has been studied already by a number of authors. A complete list of the older investigations may be found in Tigerstedt's Lehrbuch der Physiologie des Kreislaufs,[41] papers by Asher[1] and Bayliss[2] in Ergebnisse der Physiologie, and in Nagel's Handbuch der Physiologie (Hofmann[11]). The history up to November, 1914, is given by Vincent and Cameron.[43] As to more recent important investigators of this problem, we may refer to Porter,[29–34] Martin, [22–26] Ranson,[35–36] Gruber,[8,9] and their respective co-workers, also to Domitrenko[6] and Hunt.[15,16]

Even among these recent investigators there seems to be considerable difference of opinion as to what may be regarded as the usual or normal response to afferent impulses. Thus Porter

and Quinby[34] say: "It is sometimes urged that in shock the blood-pressure falls instead of rising on stimulation of afferent nerves. This abnormal reaction was observed in several of our experiments." This statement clearly involves the assumption that a rise is the normal effect, though it is recognized that the fall is a not very unusual occurrence. Vincent and Cameron[43] seem to be of opinion that the usual effect of stimulating the central end of the cut sciatic nerve is a rise, and the fall due to a pure vasomotor reflex is rather rare. So also Hunt. On the other hand, Martin and Lacey[23] having observed regularly a definite drop in blood-pressure by weak stimulation and a rise by far more strong stimulation, became doubtful of the truth of the generally accepted doctrine that pressor responses are the normal results of sensory stimulation.

These differences of opinion must be due to some factor or factors other than the strength of stimulus. Besides the factors most usually considered, such as different modes of stimulation, different nerves, different conditions of the same nerve, different narcotics, drugs, etc., there are two important considerations recently brought forward which unmistakably affect the vasomotor reflexes or complicate the problem of their elucidation.

In 1915 Vincent and Cameron[43] called attention for the first time to a fall of blood-pressure caused by increased respiratory movements. They write: "While anaesthesia is fairly complete the effect of stimulating the central end of the cut sciatic nerve is a pure and distinct rise. As the effect of the anaesthetic begins to pass off, the effect of stimulation will be a rise of blood-pressure followed by a more or less pronounced fall. Respiratory movements will now be found to have been markedly increased, and the extent of the fall of pressure appears to be at any rate proportional to the violence of the respiratory activity."

Martin and Lacey[23] investigated the influence of the interruption of the primary current at widely varying rates, but failed to notice any effect, as also did Hunt[14] in his earlier work. Only quite recently it was clearly pointed out by Gruber[8] that with the same strength of stimulus, pressor and depressor results

were obtainable by varying the rate of stimulation from 1 to 20 stimuli per second. This was later incidentally confirmed by Hunt.[16]

Thus, for the investigation of the complicated problem of vasomotor reflexes, it became very necessary to investigate each possible factor separately. In this way only would one be able to answer correctly for the normal vasomotor response.

The present investigation was undertaken for the purpose of studying some of the factors separately, of confirming previous investigations, and of trying, if possible, to reconcile contradictory views as to the conditions which determine any particular vasomotor response.

We beg to acknowledge our indebtedness to Mr. John Carmichael for his valuable assistance in all our experiments.

2. THE INFLUENCE OF RESPIRATORY MOVEMENTS UPON BLOOD-PRESSURES

It is well known that the respiratory center can easily be affected by central stimulation of sensory nerves. Thus Howell[13] writes in his Textbook of Physiology that "stimulation of any of the sensory nerves of the body may affect the rate or the amplitude of the respiratory movements." But no mention is made of the influence of these movements upon blood-pressure. The same applies to other text-books, except Starling's,[38] in which we find, "The increased respiratory movements will also aid the venous circulation and have a similar effect in increasing the systolic output," which would necessarily bring about a *rise* of blood-pressure. But, "A constant and immediate result of exaggerated respiratory movements is a fall of blood-pressure," and not a rise, as Vincent and Cameron pointed out. They found that "the extent of the fall of pressure appeared to be at any rate largely proportional to the violence of the respiratory activity," that the fall of blood-pressure was "brought about by performing rapid artificial respiration by compression of the thorax," that "deep voluntary breathing in the case of the human subject produced a regular and pronounced lowering of the blood-pressure," that "the more widely the thorax is opened

the more the fall of pressure tended to become replaced by a rise," and that the effect of artificial respiration was "a rise, and not a fall, when the animal was under curare," i.e., when a stop was put to the spontaneous respiratory movements. Thus the fall of blood-pressure as a result of increased respiratory movements seems to have been sufficiently established.

By the majority of previous observers curare was thought to be an indispensable drug in the study of the problem of vasomotor reflexes, with or without any consideration of its action on the vasomotor center itself. But we know that narcotics and other drugs are not always free from influence upon these reflexes, as pointed out by various previous investigators,[14,29,3] and, therefore, in experimental work they should be reduced to as few as possible or altogether eliminated (Vincent and Cameron). The change in character of the respiratory movements, especially their increase, becomes thus an almost unavoidable complication in the study of vasomotor reflexes when curare is not used and the narcosis is not deep enough. If this complication be left out of consideration, erroneous conclusions may be reached.

Since the appearance of Vincent and Cameron's paper several writers have referred to the influence of the increased respiratory movements. Unfortunately, they are not in complete harmony with one another. Ranson and Billingsley[35,36] say, "With stronger stimulation the greatly increased respiratory movements may no doubt play an important part in the drops in blood-pressure," but Gruber and Kretschmer[9] write that their "experiments do not support Vincent and Cameron's theory that the fall in blood-pressure is brought about by movements of respiration which interfere with the heart's activity." This latter statement seems to deny definitely the respiratory rôle upon blood-pressure. Vincent and Cameron did not positively deny that there is a true vasomotor fall of blood-pressure under certain conditions as a result of central stimulation of afferent fibers. But they insisted, and rightly, too, that many apparent vasomotor falls are really due to increased respiratory movements. We shall see later that the fall of blood-pressure with weak stimuli is a commoner occurrence than Vincent and

Cameron were inclined to believe. At any rate, the matter is so important that we have repeated the experiments to test the effects of respiration on blood-pressure.

We have stimulated electrically the central stump of several cut nerve trunks (saphenous, tibial, peroneal, sciatic, ulnar, and median) with various strengths of stimulus.

When the narcosis (with ether or chloroform) was not deep enough, the respiratory movements were always increased by strong stimulation. The most frequent response of the blood-pressure to stimulation, e.g., of the sciatic nerve, may be illustrated by figure 1.

With weak stimulation there is practically no increase of respiratory movements either in amplitude or in frequency, and the blood-pressure is either a fall or a fall followed by a more or less marked rise. When stronger stimuli are applied, the respiratory movements increase either in amplitude or in frequency, or in both, and the blood-pressure rises, instead of falling, and is followed by a marked fall. The rise of blood-pressure increases usually in proportion to the development of the strength of stimulation.

In figure 2 the anesthesia was made much deeper with the same animal as in figure 1, and stimuli of several strengths were applied to the same nerve.

There is very little increase of respiratory movements on each stimulation, and the response of blood-pressure is also small in degree. The latter, as seen from the figure, is either a fall or a rise according to the strength of stimulation, and the marked fall after the rise which is observed in figure 1 simultaneously with the increased respiration cannot be seen. This suggests at once that the marked fall accompanied by remarkably increased respiratory movements might be ascribed, at any rate, mainly, to the influence of the latter movements caused by sensory stimulation. Moreover, under brain compression it is not very difficult to stop the respiratory movements entirely, and in this case only very strong stimulation will initiate spontaneous respiratory movements. Under these conditions a fall of blood-pressure of the same character as that observed with an in-

creased respiration never occurs. Thus it would not be unreasonable to assume that figure 2 shows real vasomotor reflexes, even though weak, not complicated by the increased respiratory movements, while figure 1 represents the vasomotor reflex masked by the effects of increased respiration.

It is often very difficult or almost impossible to obtain any rise of blood-pressure when the respiratory movements are very violent. In those cases a marked fall is the only result of central stimulation of afferent fibers.

How these increased respiratory movements affect the blood-pressure was very carefully investigated by Vincent and Cameron. After pointing out several possible causes, they came to the conclusion that this fall is due to direct mechanical interference with the heart's action and with the return of the blood to the heart.

In order to confirm this theory, we opened the thorax in the middle line as did Vincent and Cameron, and found that the falls disappeared. The contrast is clearly shown in figures 3 and 4.

In these two cases the same nerve of the same animal was stimulated with the same strength of stimulus, in figure 3 in the intact animal and in figure 4 with thorax open.

In addition to opening the thorax we cut both vagi, both phrenici, and as many intercostals as possible on both sides, without obtaining very different results from those obtained by merely opening the thorax.

In a very few cases a marked fall of a similar character to that due to the increased respiratory movements, was observed in animals with thorax wide open. It was, however, soon discovered that this fall was produced by compression of the inferior vena cava by the heart which became more freely movable than before through opening the thorax. The heart fell back upon the soft-walled vein, and thus diminished the flow of blood to the right heart.

But it is certainly true that by means of almost pure vasomotor reflex, i.e., without any or with very little increase of respiratory movements one can obtain a marked fall preceded by a rise, as shown in figure 5.

Therefore we do not conclude that such a fall of blood-pressure is always produced by increased respiratory movements. But the important point for us at present is the undoubted fact that increased respiratory movements can and do cause a fall of blood-pressure, and that this fall can be easily eliminated by opening the thorax sufficiently wide.

These observations, along with various others quoted above from the paper by Vincent and Cameron both on animals and on human subjects, confirm fully their statement as to the occurrence of a fall of blood-pressure brought about by increased respiratory movements, and probably explain the nature of this fall. We believe that the increased respiratory movements caused by sensory stimulation form a very important complication which has often led to misunderstanding of the true vasomotor reflexes.

Gruber and Kretschmer, as mentioned before, deny this respiratory effect upon blood-pressure. They used a slow rate of stimulation and the fall of blood-pressure was the usual effect. But the fall is generally thought to be a result of weaker stimulation, and they do not deny that the increased respiratory movements to a certain degree cause a fall of blood-pressure when the stimulus is strong enough as to produce them.

Our experiments were made on thirty-three dogs.

3. THE EFFECT OF THE STRENGTH OF THE STIMULUS UPON VASOMOTOR REFLEXES

After repeated experiments by numerous investigators, the generally accepted view as to the effect upon the vasomotor reflexes of different strengths of stimulus seems to coincide with Knoll's[19] original statement, i.e., that a depressor effect is usually the result of a weak stimulation, while a pressor effect follows, as a rule, a stronger stimulation. Reid Hunt[14] pointed out that weak stimulation was one of the methods of obtaining a reflex fall of blood-pressure, and Vincent and Cameron noticed the same fact.

Among more exhaustive investigations on this point we should refer to those by Porter,[29–34] Martin,[22–26,40] and their respective

co-workers. The former writer seems to regard a rise of blood-pressure as the normal vasomotor response, while the latter holds a different view. Martin and Lacey's[23] experiments were conducted on cats either under brain pithing, decerebration or brain compression, or under ether or urethane. The nerves stimulated were the sciatic, radial, median, ulnar, and saphenous. The results of their experiments were very definite. "In every one of the experiments the stimulation was repeated many times over a range of stimuli from the threshold value to three or four times the threshold. Well-marked drops of pressure followed all such stimulations," save in one exceptional case. Thresholds for pressor reflexes were much higher than those for depressor reflexes. Thus the experiments of these workers support Knoll's statement.

Our own experiments consisted in stimulating various nerves (sciatic, tibial, peroneal, saphenous, median, ulnar, and vagus) with induction shocks on dogs under ether, chloroform, and brain compression. As to the method, we have to mention that the different effects of weak and strong currents, respectively, were satisfactorily attained by means of sliding the secondary coil up to or away from the primary, but that on many occasions more than one battery was used to obtain a stronger stimulus. The rate of stimulation was 38 to 54 in a second.

The fall of blood-pressure due to the increased respiratory movements being taken into consideration, the main results of our experiments may be summarized as in the following table:

ANAESTHETICS	FALL WITH WEAK STIMULATION. RISE WITH STRONG STIMULATION	FALL WITH WEAK AND STRONG STIMULATION	RISE WAS ONLY RESULT OF STIMULATION
Ether	20	2	4
Chloroform	14	4	4
Brain compression	12	2	0
Total	46	8	8

The term 'fall' in the table comprises also a fall followed by a rise and 'rise' also a rise followed by a fall.

In forty-six cases out of sixty-two in total, weak stimulation produced a fall or a fall followed by a rise, and strong stimulation caused a rise or a rise followed by a fall. A typical response is shown in figure 6.

The animal was under ether and the thorax was very wide open in the middle line in order to eliminate the disturbance from increased respiratory movements. Figure 7 shows a similar response under chloroform.

In the remaining sixteen cases the response was either a fall or a rise through all strengths of stimulation which we used, and the different effects with weak and strong stimulation were not observable.

Thus it does not seem to us unreasonable to conclude that weak stimulation of the central stump of the cut nerve produces usually a fall of blood-pressure and a strong stimulation produces usually a rise.

From these conclusions it may naturally be understood that from the threshold of stimulation up to a certain point the fall of blood-pressure increases with the development of the strength of stimulus, and then the fall gradually decreases until a neutral point is reached, where the vasoconstriction and dilatation just counterbalance each other, and finally the rise appears, which increases usually with the increase of the strength of stimulus, but cannot continue very long, since powerful stimuli would elicit vigorous reflex movements of the animal and obscure the true vasomotor reactions unless indeed the animals were deeply under curare. As we have been unable so far to find any attempt by previous investigators except Stiles and Martin[40] to describe this rather peculiar course of vasomotor responses, we though it worth while to emphasize it in this place (fig. 8).

In our experiments we have employed also stimuli of other kinds than electric induction shocks, namely, mechanical, thermal, and chemical. In this series thirty-eight out of sixty-seven stimulations were effective, and of these thirty-five caused a fall of blood-pressure and only three produced a rise. As the calibration of these stimuli was not so practicable as with induction shocks, we cannot draw any very positive conclusions, but we

are inclined to believe that a greater number of pressor responses could be obtained if we could improve the method of stimulation so that the sensory fibers might be stimulated more strongly.

It thus appears from our experiments that the depressor effect of weak stimuli is much more common than Vincent and Cameron thought, though these observers were careful not to deny its occurrence. Reid Hunt,[16] in a recent paper, seems to have had the same difficulty that Vincent and Cameron encountered in obtaining the depressor effect of weak stimulation, which he ascribed to the different frequency of stimulation they employed.

The fact that a weak stimulation of a sensory nerve causes, as a rule, a reflex fall of blood-pressure and a strong stimulation a reflex rise, together with the statement of Bayliss[3] that the orthodox effect due to the stimulation of the depressor nerve (nerve of Cyon[5]) can be converted into a rise by the action of strychnine, led us to inquire whether a pressor response could be obtained by strong stimulation of the depressor nerve. So far as our results inform us, neither such a strong current as would injure the nerve nor induction shocks up to eighty per second frequency could reverse the depressor response. The response to the stimulation after injection of strychnine was sometimes increased and sometimes decreased, but the reversal of the response did not appear in our experiments even with a dose which caused general convulsions on weak stimulation.

4. THE INFLUENCE OF THE FREQUENCY OF STIMULATION UPON VASOMOTOR REFLEXES

That the frequency of stimulation has a certain effect upon vasomotor reactions seems to have been known to the older investigators. In 1883 Kronecker and Nicolaides[20] noticed that the vasomotor centers could more easily be affected by changing the frequency of stimulation than by changing its strength. They write: "One can never attain such a strong vasoconstriction by increasing the intensity of the stimulating current as by increasing the frequency of the current of moderate intensity." We have not been able to consult the original paper of these writers.

From a reference in Ergebnisse der Physiologie by Asher,[1] it is not clear whether the stimulation was directly upon the nerve centers or reflexly through afferent nerves.

But the credit of pointing out clearly that the frequency of stimulation has an effect upon vasomotor reflexes must be ascribed to Gruber.[8] This writer remarks: "That summation takes place with rapid rates of stimulation is undisputable, but it does not seem probable where the strength is more than 400 times threshold that the phenomenon of summation can explain the different effect obtained with these rates of 1 per two seconds and 20 per second interruptions." The similar effect of frequency of stimulation was afterward proved incidentally by Reid Hunt,[16] who considers it convenient to use the infrequent rate of stimulus to obtain a reflex fall of blood-pressure.

Our experiments on this subject have been carried out on dogs with rates of stimuli of 1, 2, 5, 10, 20, 40, and 80 per second upon various nerves, under chloroform and curare or under brain compression. Though our results were not so conclusive as those obtained by Gruber (in fifteen out of forty stimulations similar results to his were obtained), still we do not hesitate to ascribe an important rôle to the frequency of stimulation. According to Martin's[22] investigations, the intensity of stimulation in Z-units is directly proportional to that of the current in the primary circuit. We arranged the apparatus in such a way as to get a current of a certain strength and one ten times stronger as we desired. With the former current we obtained a fall by stimulating five times per second, and a distinct rise by stimulating ten times per second, while with the latter current we observed a fall with the rate of stimulus one per second and a rise with five per second stimulations. A selected record is shown in figure 9, where one and the same nerve was stimulated with the same intensity but with different frequency.

Much more remarkable were the rates of stimulation at which the maximum pressor response was reached.

	RATE OF STIMULI PER SECOND						
	1	2	5	10	20	40	80
Number of experiments whose maximum pressor response was reached at the rate of stimuli mentioned above	0	0	0	4	18	12	4

As is seen from the table, in 78.9 per cent the maximum response is reached between twenty to forty per second stimulation, and in one-third at the rate of forty per second. Beyond these points the effect increased only in four cases. This phenomenon may be seen also in figure 9.

Kronecker and Nicolaides[20] observed the fact that the effect of stimulation of the vasomotor centers increased with the frequency of stimuli up to twenty to thirty per second, but not beyond this point. Tur[42] also pointed out that the effect of stimulation of the lingual nerve increased until the stimuli reached forty per second, beyond which, however, the effect diminished. These observations coincide fairly well with our own.

5. EFFECTS UPON VASOMOTOR REFLEXES OF STIMULATING NERVE TRUNKS OF DIFFERENT CATEGORIES (SENSORY, MOTOR, AND MIXED NERVES) AND OF DIFFERENT SIZES

According to the investigations of some authors, different nerves, apart altogether from the depressor nerve, respond differently to central stimulation. Hofmann,[11] in Nagel's Handbuch, says: "There are single nerves, which for the most part (glossopharyngeal) are depressor, and others which are exclusively (splanchnic) or preponderatingly (sciatic, facial, infraorbital, cervical nerves) pressor." Vincent and Cameron studied the effect of stimulating the main trunk of the sciatic, as well as its common peroneal, lateral cutaneous, and purely muscular branches, the saphenous, median of axilla, the hypoglossal, the glossopharyngeal, the superior laryngeal, and the vagus. But the different nerves all produced similar or comparable results on the blood-pressure. They were strongly tempted to the hypothesis that an equivalent stimulation of a roughly equal number of afferent fibers will yield similar reflexes.

Our experiments also have led to the conclusion that there is no essential qualitative difference between the various nerves subjected to stimulation (sciatic, tibial, peroneal, median, ulnar). A possible exception may be made in the case of the saphenous. It may be that there is a greater tendency to a fall on stimulating this nerve than in the case of others. Whatever the nerve may be, whether a purely sensory nerve, as the saphenous; a mixed nerve, as the sciatic, peroneal, tibial, ulnar, or median, or a purely motor nerve, such as a muscular branch of the femoral, the course of response to weak and strong stimulation was in most cases a fall and a rise as already described in section 3.

A few examples are quoted in tabular form where a selected purely sensory nerve and a purely motor or a mixed nerve apparently of the same size were stimulated in turn under entirely similar conditions.

Sensory and motor nerves compared

ARRANGEMENTS OF STIMULATION	RIGHT SAPHENOUS	BRANCH OF RIGHT FEMORALIS
1 battery coil at 30 cm. 15 seconds...	−4 mm. Hg.	0, mm. Hg.
1 battery coil at 25 cm. 15 seconds...	−10 mm. Hg.	−1, +2 mm. Hg.
1 battery coil at 20 cm. 15 seconds...	−10, +2 mm. Hg.	−4, +2 mm. Hg.
1 battery coil at 15 cm. 15 seconds...	+4, −12 mm. Hg.	+4, 0 mm. Hg.
1 battery coil at 10 cm. 15 seconds...	+6, −14 mm. Hg.	+8, −2 mm. Hg.
1 battery coil at 5 cm. 15 seconds...	+16, −18 mm. Hg.	+14, −12 mm. Hg.
1 battery coil at 0 cm. 15 seconds...	+22, −14 mm. Hg.	+26, −14 mm. Hg.

(From experiment 47. Under brain compression.)

The sign '−' means a pure fall of blood-pressure, '+' a pure rise, and '−, +' or '+, −' a mixed response, namely, a fall followed by a rise or a rise followed by a fall, respectively.

Sensory and mixed nerves compared

ARRANGEMENTS OF STIMULATION	RIGHT SAPHENOUS	RIGHT PERONEAL
1 battery coil at 30 cm. 15 seconds...	0 mm. Hg.	0 mm. Hg.
1 battery coil at 25 cm. 15 seconds...	−6, 0 mm. Hg.	−1, 0 mm. Hg.
1 battery coil at 20 cm. 15 seconds...	+2, −10 mm. Hg.	−4, +4 mm. Hg.
1 battery coil at 15 cm. 15 seconds...	+6, −12 mm. Hg.	+2, −6 mm. Hg.
1 battery coil at 10 cm. 15 seconds...	+8, −12 mm. Hg.	+14, −10 mm. Hg.
1 battery coil at 5 cm. 15 seconds...	+22, −10 mm. Hg.	+20, −14 mm. Hg.
1 battery coil at 0 cm. 15 seconds...	+22, −12 mm. Hg.	+22, −20 mm. Hg.

(From experiment 47. Under brain compression.)

With the increase of the strength of stimulus the respiratory movements also increased, though not very markedly, and therefore some of the falls following the rise on stronger stimulation might have been more or less due to this complication. But in the main it seems that the purely sensory nerves have somewhat lower threshold than other kinds of nerves (fig. 10).

Whether this is due to a large number of afferent fibers contained in the sensory nerve than in those of the other kinds of the same size could only be decided by more numerous experiments and more elaborate methods than those we have employed, as, for example, the measurement of resistance of each nerve and more satisfactory methods of controlling the intensity of stimulation in each case.

In connection with the problem as to different kinds of nerves we have studied the influence of the size of the nerve upon vasomotor reflexes. The hypothesis of Vincent and Cameron is quoted at the beginning of this section. A similar problem was taken up also by Stiles and Martin,[40] who compared the effect of stimulating two nerve paths at the same time with that of exciting each by itself. They found that "stimulation of two afferent paths at the same time has often a more marked vasomotor effect than the stimulation of either path alone with an equivalent strength of current. The degree of summation was only moderate." This shows that the stimulation of a larger number of afferent fibers will produce often a more marked effect than that of few fibers.

We stimulated two nerves of the same category but of different sizes separately one after another under conditions as similar as possible, a different number of afferent fibers being assumed to be present in the nerves of different sizes.

The results may be represented as follows, page 369.

These few examples show that the results were not very conclusive. We can say only so far with some confidence that when the responses were in the same sense, i.e., when the fall or the rise was the result of corresponding equivalent stimulations, the reflex change of blood-pressure was on the whole more marked with the nerve of larger size than with those of smaller size (fig. 11).

Mixed nerves compared with each other

ARRANGEMENTS OF STIMULATION	RIGHT SCIATIC	RIGHT PERONEAL
1 battery coil at 30 cm. 15 seconds...	0 mm. Hg.	0 mm. Hg.
1 battery coil at 25 cm. 15 seconds...	−6, 4 mm. Hg.	−10, 0 mm. Hg.
1 battery coil at 20 cm. 15 seconds...	−14, 0 mm. Hg.	−8, 4 mm. Hg.
1 battery coil at 15 cm. 15 seconds...	12, −22 mm. Hg.	8, −6 mm. Hg.
1 battery coil at 10 cm. 15 seconds...	18, −16 mm. Hg.	12, −10 mm. Hg.
	RIGHT SCIATIC	RIGHT TIBIAL
1 battery coil at 25 cm. 15 seconds...	−2, 2 mm. Hg.	−2, 2 mm. Hg.
1 battery coil at 30 cm. 15 seconds...	−6, 4 mm. Hg.	−2, 0 mm. Hg.
1 battery coil at 15 cm. 15 seconds...	16, −18 mm. Hg.	10, −8 mm. Hg.
1 battery coil at 10 cm. 15 seconds...	22, −22 mm. Hg.	20, −16 mm. Hg.

(From experiment 47. Under brain compression.)

Sensory nerves compared with each other

ARRANGEMENTS OF STIMULATION	RIGHT SAPHENOUS	A BRANCH OF THE RIGHT SAPHENOUS
1 battery coil at 25 cm. 10 seconds...	0 mm. Hg.	0 mm. Hg.
1 battery coil at 20 cm. 10 seconds...	−6, 0 mm. Hg.	0 mm. Hg.
1 battery coil at 15 cm. 10 seconds...	−18, 0 mm. Hg.	−2, 0 mm. Hg.
1 battery coil at 10 cm. 10 seconds...	−22, 0 mm. Hg.	−8, 0 mm. Hg.
1 battery coil at 5 cm. 10 seconds...	−18, 0 mm. Hg.	+2, 0 mm. Hg.
1 battery coil at 0 cm. 10 seconds...	+4, 0 mm. Hg.	−4, 0 mm. Hg.
	RIGHT SAPHENOUS	A BRANCH OF THE RIGHT SAPHENOUS
1 battery coil at 25 cm. 10 seconds...	0 mm. Hg.	0 mm. Hg.
1 battery coil at 20 cm. 10 seconds...	−4, 0 mm. Hg.	0 mm. Hg.
1 battery coil at 15 cm. 10 seconds...	−8 ,0 mm. Hg.	0 mm. Hg.
1 battery coil at 10 cm. 10 seconds...	−4, 0 mm. Hg.	−4, 0 mm. Hg.
1 battery coil at 5 cm. 10 seconds...	+4, 0 mm. Hg.	−4, 0 mm. Hg.
1 battery coil at 0 cm. 10 seconds...	+2, 0 mm. Hg.	−6, 0 mm. Hg.

(From experiment 48. Under brain compression.)

These conclusions coincide with the experience of Stiles and Martin and lend some support to the hypothesis of Vincent and Cameron.

It may not be amiss to add to these conclusions that in few cases when the stimuli were very strong the smaller nerve reacted more vigorously than the larger one, which phenomenon may perhaps be explained partly by different resistances of different-sized nerves to currents of similar strength.

Thus, so far as our experiments go, we are inclined to conclude provisionally that among nerves of different categories there are no essential qualitative differences of response, and the greater the number of afferent fibers stimulated, the more marked is the response of blood-pressure within a limited range of strength of stimulation.

6. VASOMOTOR REFLEXES FROM NERVE TERMINATIONS

Several investigators have stimulated the nerve terminals instead of the nerve trunk itself.

When we apply a stimulus to a surface such as the skin we should bear in mind that we may actually be stimulating either the end-organs alone or these structures as well as the nerve fibers, according to the mode of stimulation. Any physiologically appropriate stimulus, though mild, applied to the end-organs would give rise to more highly effective impulses than inappropriate ones. Thus the study of vasomotor reflexes in response to stimulation of the sense organ with its most appropriate stimulus is highly desirable. But even with other kinds of stimulation we may learn much that is valuable, because any stimulus which plays a part in our normal daily life comes usually through the end-organs on the outer or inner surface of the body, and not by way of exposed nerve trunks, as in the foregoing experiments.

The skin, the mucous membrane of the nose, muscles, the intestine, and other abdominal organs were employed frequently by previous investigators. We have selected the skin, muscles, and the intestine as representative of regions containing different modes of nerve endings. The stimuli used were mechanical (incision, scratching, pinching, kneading), thermal (hot or boiling water and cold water or lumps of ice), chemical (10 per cent solution of sulphuric acid), and electrical (induction shocks of various strengths). The animals (dogs) were under ether, chloroform, or brain compression.

The results of a first series are presented in the following tables:

Mechanical stimulation of the skin

ANESTHESIA	STIMULATED PORTION	MODE OF STIMULATION	REFLEX RESPONSE OF BLOOD-PRESSURE		
			No effect	Fall	Rise
Ether	L. inn. thigh	Pinching	0	2	0
	R. inn. thigh	Scratching	2	5	0
	R. inn. thigh	Incision	0	6	0
Chloroform	L. inn. thigh	Pinching	1	1	0
	R. inn. thigh	Scratching	2	3	0
	Abdomen Over saphenous, ulnar, and sciatic nerves	Incision	3	4	0
Brain compression	L. inn. thigh	Scratching	0	2	0
	R. inn. thigh	Incision	0	3	0
Total			8	26	0

Thermal stimulation of the skin

Ether	L. inn. thigh	65°C.—boiling water	0	5	0
	L. inn. thigh	Ice—15°C. water	3	0	0
Chloroform	L. inn. thigh	Boiling water	2	3	0
	L. inn. thigh	Ice	0	1	0
Brain compression	L. inn. thigh	Boiling water	0	1	1
	L. inn. thigh	Ice	2	0	0
Total			7	10	1

Electrical stimulation of the skin

Ether	L. inn. thigh	Strong induction shocks	8	5	0
Chloroform	L. inn. thigh	Strong induction shocks	4	2	0
Brain compression	L. inn. thigh	Strong induction shocks	4	2	0
Total			16	9	0

As is clear from the tables, almost every stimulation in this series, produced a reflex fall of blood-pressure, and no significant qualitative difference is observable either with different modes of stimulation or with different methods of anaesthesia or with different portions of the skin.

That this statement is applicable almost without any modification to the results of stimulation of muscles and intestine will immediately be understood from the tables on page 373.

Thus it is fairly clear that the stimulation of nerve terminals in the skin, muscles, and the intestine produces usually a reflex fall of blood-pressure, as was reported by Vincent and Cameron.

But the threshold of stimulation for the nerve-terminals in the skin is very much higher than that for the exposed nerve-trunks. Thus a stimulus which is to be reckoned a strong one for the exposed nerve-trunk is to be considered a weak one for the surface of this skin. This fact explains the previously described results. So far the effects have all been those of a weak stimulation, namely, a fall of the blood-pressure.

If, now, we take steps to secure a considerably greater amount of stimulation by simultaneous scratching of large areas in different regions, it is not difficult to satisfy oneself that the same general law applies for the nerve-terminals as for one exposed nerve-trunk. Thus, if we scratch a limited area with a moderate degree of vigor, we get a fall, while more violent application of the instruments to a large area, will give a rise (fig. 19).

In the last section we compared the effects of stimulating two nerves of the same category but of different sizes, and showed that the nerves of greater size usually surpass those of smaller size in their power of evoking vasomotor reflexes, and referred to Vincent and Cameron's hypothesis that the number of afferent nerve fibers is an important factor. In our stimulation of nerve endings, as a rule, we could only apply the stimulations to a small portion of the surface. Now the nerve fibers spread widely from the nerve trunk, and the stimulation of a nerve would be equivalent to the stimulation of the entire surface to which the nerve is distributed. In other words, the stimulation of a small portion, e.g., of the skin, corresponds to that of a

Mechanical stimulation of muscles

ANESTHESIA	STIMULATED MUSCLE	MODE OF STIMULATION	REFLEX CHANGE OF BLOOD-PRESSURE		
			No effect	Fall	Rise
Ether	R. add. mag.	Scratching	0	1	0
	R. sartor.	Scratching	0	2	0
	R. sartor.	Kneading	0	3	0
	L. semitend.	Scratching	3	0	0
Chloroform	R. add. mag. or 1 semitend.	Scratching	4	0	0
Total			7	15	0

Stimulation of the intestine

Ether	Small intestine	Kneading	0	3	0
	Interior surface of small intestine	Induction shocks	1	1	0
	Interior surface of small intestine	Pinching	0	1	0
	Interior surface of small intestine	Boiling water applied	1	1	0
	Small intestine	Distension	0	4	0
Chloroform	Small intestine	Kneading	0	2	1
Brain compression	Small intestine	Kneading	0	4	0
	Interior surface of small intestine	Induction shocks	0	1	2
	Interior surface of small intestine	Scratching	0	1	0
	Interior surface of small intestine	Boiling water applied	0	1	0
	Interior surface of small intestine	Ice piece applied	0	1	0
Total			2	20	3

small number of sensory nerve fibers. If the stimulation of a few fibers be the equivalent of a weak current, the fall of blood-pressure caused by stimulating the nerve terminals may be ascribed to the fact that we are stimulating only a few fibers.

Under morphia and curare a rise of blood-pressure is more easily obtained than a fall on stimulation of nerve-endings. But under morphia at any rate it is not difficult to obtain a rise with a strong stimulus and a fall with a weak one (fig. 19). Gaskell's[7] discovery that in mammals "A large dose of curare will remove both the contraction of the muscle and the dilatation of its blood-vessels upon stimulation of the nerve," may possibly account for the greater tendency towards a rise when the animal is under this drug.

The paralysis of the vasodilator nerves by curare seems to necessitate the taking of certain precautions in interpretation of the results of experiments.

It seems probable that if it were found possible to increase very considerably the energy and extent of the stimulation in the cases of kneading of muscle and of the intestine, we should have to record a rise of pressure instead of the fall with which we are familiar.

7. THE INFLUENCE OF THE DUCTLESS GLANDS UPON VASOMOTOR REFLEXES

The extracts of some of the ductless glands (adrenal body, thyroid, and pituitary) have been alleged to affect the vasomotor irritability on one way or another.[17,43,12,27,28] Since the results of the previous investigations are not conclusive, we thought it might be worth while to investigate the matter again. It is to be feared that our experiments are not much more convincing than those of previous workers on this subject.

The change of blood-pressure (augmentation or diminution) due to the injection of the extracts of these glands is an undesirable complication. In cases where an augmented blood-pressure is the result, as with adrenin and pituitrin, the decreased pressor reaction to the stimulation of a nerve may most properly be ascribed to the diminished response of the already more or

less contracted blood-vessels, or possibly to the additional contraction of the blood-vessels of the small areas other than those previously affected by the drug.

But the comparison of the vasomotor reflexes before and after the injection of adrenin ("adrenalin" Parke, Davis & Co.) seems to show that the pressor reflex is slightly decreased in the latter case. The results of Hoskins and Rowley were similar and more definite. The elimination of the function of the suprarenal glands by tying them off gave no clear results.*

The injection of thyroidin (Parke, Davis & Co.) and the extirpation of both thyroid glands do not appear to have any distinct influence upon vasomotor reflexes.

Pituitrin (Parke, Davis & Co., surgical) showed scarcely any significant results.

All these experiments were performed on dogs under brain compression for the purpose of excluding any influences from the increased respiratory movements and those of anaesthetics and other drugs.

8. THE QUESTION AS TO WHICH VASCULAR AREAS ARE CONSTRICTED OR DILATED ON CENTRAL STIMULATION OF SOMATIC NERVES

The fall of blood-pressure produced by stimulation of the depressor nerve is effected chiefly by dilatation of the splanchnic area,[21] though, as Bayliss has shown, the vessels of the limbs, head, and neck also partake in the relaxation. The latter writer showed also that the rise of blood-pressure on stimulation of the central stump of the splanchnic (?) nerve was, for the most part, due to the constriction in the splanchnic area. The reflex rise of blood-pressure due to the stimulation of the

*That is to say, when the nerves to the limb are intact. In the denervated limb there is a very important difference according to whether or no the adrenal bodies are eliminated. Mr. Pearlman and myself have recently found that when the central end of the sciatic is stimulated in such a way as to give a pressor response the intact limb follows passively the blood-pressure while the denervated limb constricts. After removal of the adrenal bodies the denervated limb also dilates. These results are explained more fully in a paper about to be published in 'Endocrinology.'—S. V.

sensory nerves of the skin, too, depends mainly on the constriction of the blood-vessels in the same area,[38] and Hofmann[11] writes: "The rise of blood-pressure on stimulation of sensory nerves is produced by the constriction of the blood-vessels of abdominal organs as in asphyxia. At the same time the blood-vessels of the brain, skin, and muscles dilate, as a rule, and an increase of the volume of limbs takes place." Thus the splanchnic area plays a principal part in the reflex changes of blood-pressure on stimulation of the somatic as well as the splanchnic nerves.

We have made some experiments on this point, and can confirm the above statements. The dogs had both vagi cut and were under morphia and curare or brain compression, and the sciatic or saphenous nerve was stimulated with induction shocks. The volume changes of the limbs (hind and fore) and of the abdominal organs (small intestine, kidney, and spleen) were recorded.

The rise of blood-pressure, when sufficiently high, was always accompanied by a remarkable diminution of the volumes of abdominal organs and a pronounced dilatation of limbs (fig. 18).

The pronounced fall of blood-pressure with weak stimulation when the animal was under brain compression was seen to be accompanied by a distinct increase of the volume of the intestine. Thus it appears clear that a reflex rise and fall of blood-pressure on stimulation of a somatic nerve (sciatic, saphenous) is brought about chiefly by constriction and dilatation of the blood-vessels in the splanchnic area.

9. SUMMARY

1. In dogs under ether or chloroform, stimulation of sensory nerves (saphenous, tibial, peroneal, sciatic, ulnar, and median) causes usually increased respiratory movements when narcosis is not profound or curare is not employed. These increased movements produce a fall of blood-pressure, and when they are very violent, one cannot obtain any pressor reflex even with a strong stimulation. When much increased respiratory movements are prevented by very deep narcosis or brain compression, fall of

blood-pressure due to this cause does not occur. Mechanical interference with the circulation as a result of the increased movements of the thoracic walls seems to be the main cause of this fall, since it can be eliminated by opening the thorax. When this complication is not taken into careful consideration the results of vasomotor experiments are liable to be misinterpreted.

2. In dogs under ether, chloroform, or brain compression, a weak stimulation of the central end of the cut nerves (sciatic, saphenous, tibial, peroneal, median, ulnar, and vagus) produces usually a fall and a strong stimulation, a rise of blood-pressure. With a gradual increase of the strength of stimulus up from the threshold, the reflex fall of blood-pressure first increases, then decreases, and gradually becomes converted into a rise, passing through a neutral point. We have failed to obtain a pressor effect by the strongest stimulation of the depressor nerve of Cyon.

3. The frequency of stimulation has an effect upon vasomotor reflexes. With a rapid rate of stimulation a rise is obtained and with a slow rate of stimulation in many cases a fall of blood-pressure. Of the different rates of stimulation we employed (one to eighty per second), the maximum pressor response is reached at twenty to forty per second.

4. No essential qualitative difference was found among various nerves (sciatic, tibial, peroneal, median, ulnar, branch of femoral nerve) subjected to stimulation. The saphenous nerve has a greater tendency to give a fall than those above mentioned. A purely sensory nerve seems to have a somewhat lower threshold than other kinds of nerves. Between nerves of the same category but of different sizes, the larger one produces usually a more marked response within a limited range of the strength of stimulation.

5. When the animal is under ether, chloroform, or brain compression, stimulation (mechanical, thermal, chemical, and electrical) of nerve terminations, such as those in the skin, muscles, and the intestine, causes a fall of blood-pressure in the great majority of cases, but violent or extensive stimulations of the

skin produce a rise. Under morphia and curare, on the contrary, a rise is a usual response, due clearly to a specific pharmacodynamical influence of these drugs. But under morphia a weak stimulus will produce a fall.

6. The influence of the ductless glands (adrenal, thyroid, and pituitary) upon vasomotor reflexes is not clear.* The injection of adrenin, thyroidin, and pituitrin and tying off or extirpation of the glands produced in our experiments no distinct effect.

7. The reflex change (fall or rise) of blood-pressure on stimulation of the somatic nerves (sciatic, saphenous) is produced chiefly by the dilatation or constriction of the blood-vessels in the splanchnic area, as in the cases of the stimulation of the splanchnic and the depressor nerve.

*See footnote, page 375.

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PLATES

PLATE 1

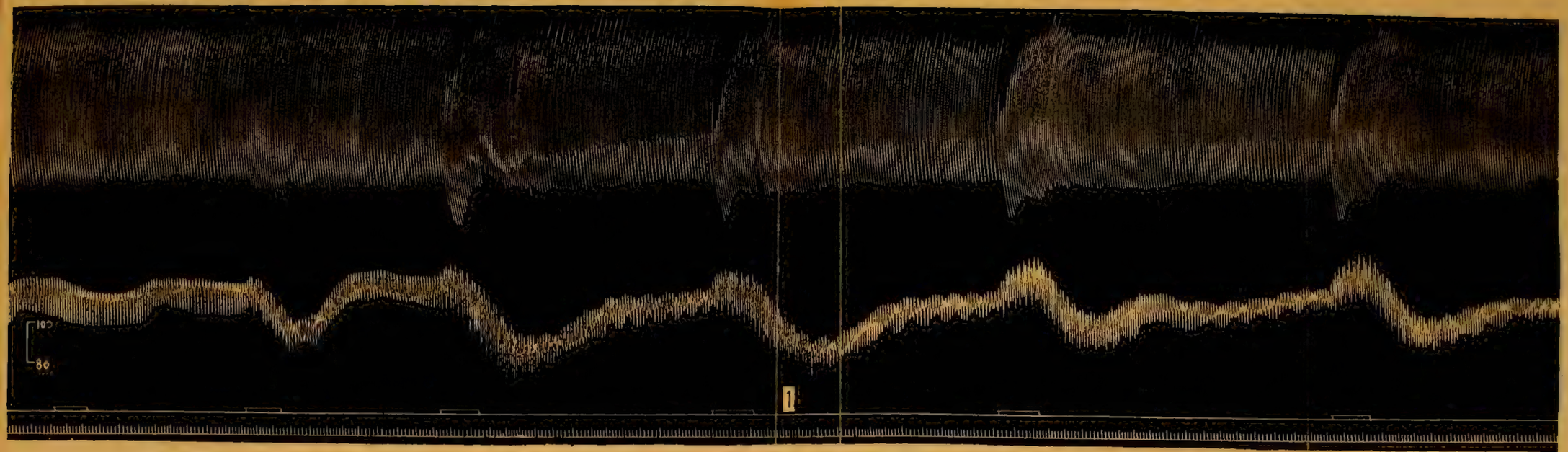
EXPLANATION OF FIGURES

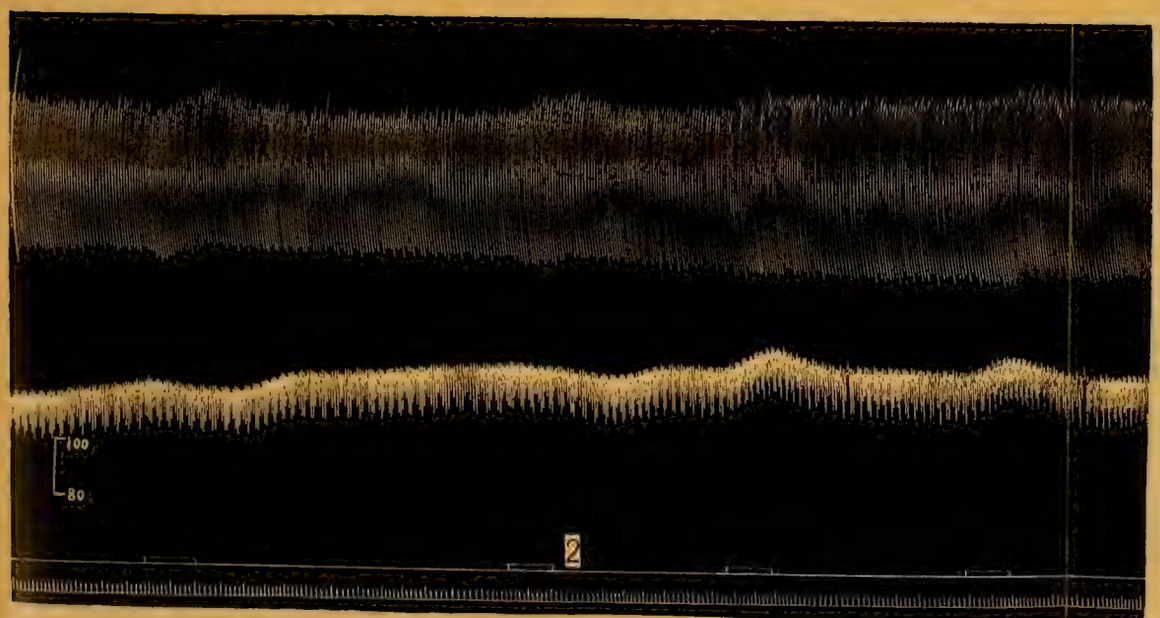
Fig. 1 The effect of increased respiratory movements upon vasomotor reflexes. Bitch. 9 kilos. 10/5/1918. Ether. The left sciatic nerve was stimulated at intervals, the stimulus increasing from left to right. Upper curve, respiratory movements. Lower curve, blood-pressure. Base line is that of zero pressure, with periods of stimulation. The height of the blood-pressure in mm. Hg. is indicated by the cm. measured out and numbered. Time in seconds. For further explanation see text.

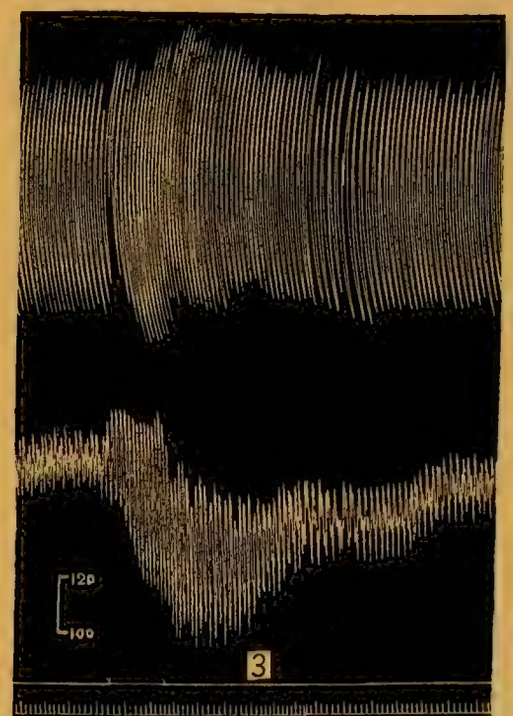
Fig. 2 Increased respiratory movements prevented by very deep narcosis. Same bitch as in figure 1. For explanation see text.

Fig. 3 Effect of increased respiratory movements upon blood-pressure. Bitch. 14 kilos. 30/5/1918. Ether. Thorax intact. The left sciatic nerve was stimulated. The result is a marked fall of blood-pressure.

Fig. 4 Effect of increased respiratory movements upon blood-pressure prevented by opening the thorax. Same bitch as in figure 3. Thorax wide open in the middle line. The same nerve was stimulated with the same strength of stimulus as in figure 3. The result is a marked rise of blood-pressure.


100
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1


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80
2


120
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3

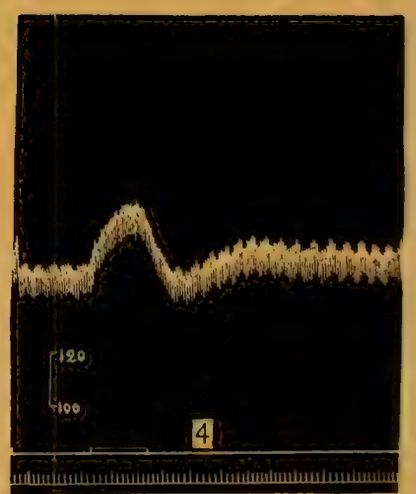

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PLATE 2

EXPLANATION OF FIGURES

Fig. 5 A marked fall of blood-pressure which is apparently not due to increased respiratory movements. Dog. 12 kilos. 9/5/1918. Ether. The left sciatic nerve was stimulated.

Fig. 6 An example of vasomotor reflexes upon weak (left) and strong (right) stimulations. Same bitch as in figure 3. Thorax was very wide open in the middle line to eliminate the influence from increased respiratory movements. A weak stimulation caused a fall and a strong stimulation caused a rise of blood-pressure.

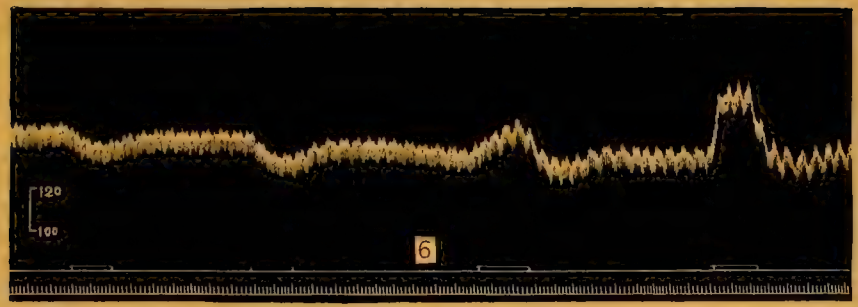
Fig. 7 Vasomotor reflexes under chloroform. Bitch. 7 kilos. 28/5/1918. Thorax wide open. A weak stimulation produced a fall (left) and a strong stimulation produced a rise (right) of blood-pressure.

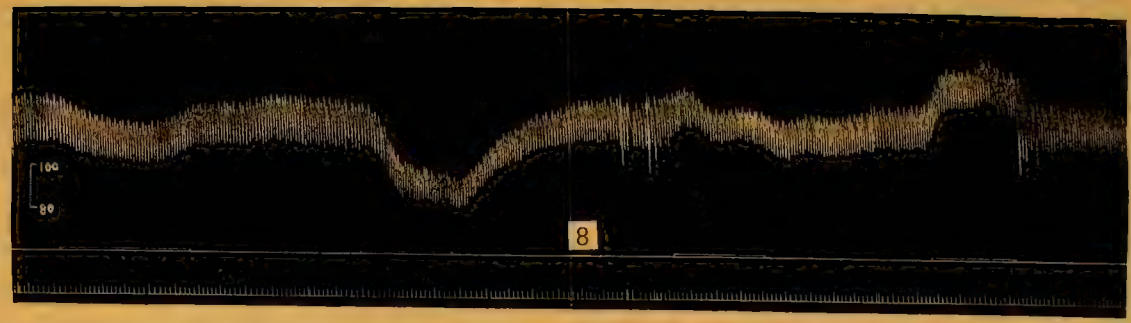
Fig. 8 Effects of weak and strong stimuli, respectively, under brain compression. Dog. 18 kilos. 15/8/1918. Brain compression and artificial respiration. Right ulnar nerve stimulated. The fall of blood-pressure increased at first with the development of the strength of stimulus and then passed over to a rise crossing a neutral point.

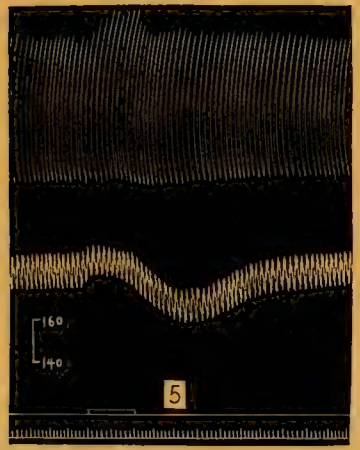
Fig. 9 Effect of frequency of stimulation upon vasomotor reflexes. Bitch. 13 kilos. 18/7/1918. Chloroform and curare. Right saphenous nerve was stimulated. The frequency employed 1, 2, 5, 10, 20, 40, and 80 per second, respectively, from left to right. The one per second stimulation caused a fall, the two per second stimulation showed practically no effect, and the other stimulations produced a rise. The maximum pressor response was reached at forty per second stimulation in this case.

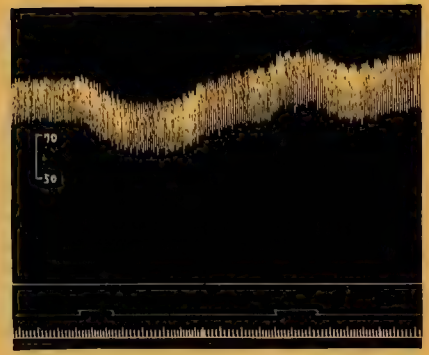
Fig. 10 Stimulation of a sensory (saphenous) and a motor (a branch of the femoral) nerve. Dog. 9 kilos. 10/10/1918. Brain compression and artificial respiration. Stimulation of a sensory nerve gave a more pronounced fall than that of a motor nerve.

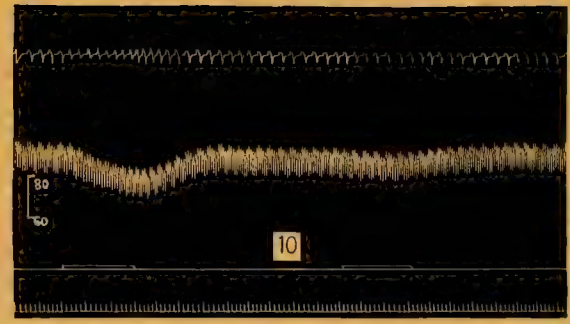
Fig. 11 Stimulation of nerves of the same category but of different sizes. Same dog as in figure 10. The stimulation of a larger nerve (sciatic) produced a more marked response than that of a smaller nerve (peroneal).

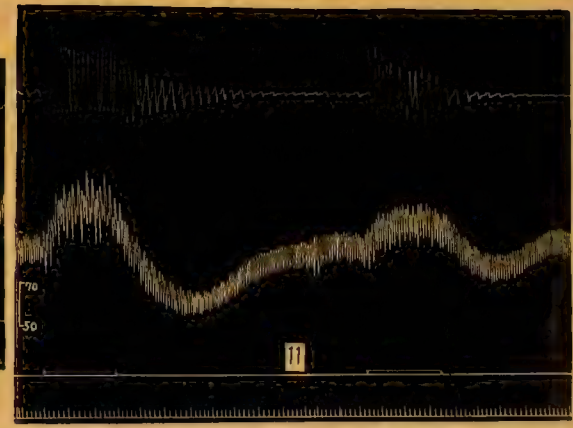


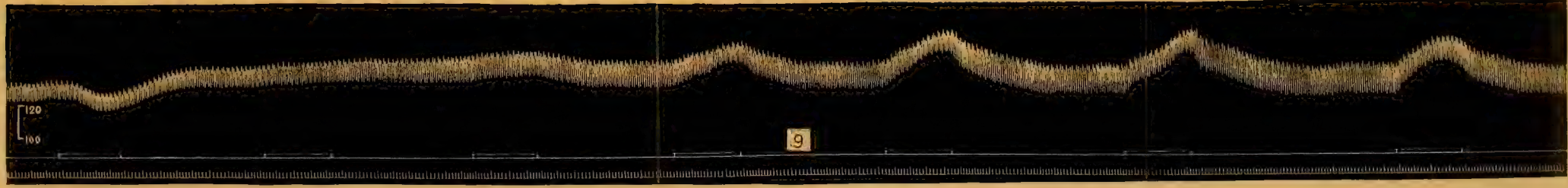












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PLATE 3

EXPLANATION OF FIGURES

Fig. 12 Scratching of the skin. Dog. 12 kilos. 9/5/1918. Ether. A marked fall of blood-pressure. Respiratory movements practically unaffected.

Fig. 13 Application of heat (boiling water) on the skin. Dog. 10 kilos. 25/10/1918. Brain compression and artificial respiration. A fairly marked fall of blood-pressure.

Fig. 14 Electrical (strong) stimulation of the skin. Dog. 11 kilos. 14/5/1918. Ether. A very marked fall of blood-pressure. Respiratory movements affected very slightly.

Fig. 15 Scratching of muscle (right sartorius). Bitch. 10 kilos. 22/10/1918. Ether. A fairly marked fall of blood-pressure. Respiratory movements show no increase.

Fig. 16 Kneading of muscles (lateral muscles of the right thigh). Dog. 10 kilos. 25/10/1918. Brain compression and artificial respiration. Gentle kneading produced a pure fall (left) and violent kneading a fall followed by a rise (right). Artificial respiratory movements affected mechanically by the manipulation.

Fig. 17 Kneading of the small intestine. Same dog as in figure 16. A fairly marked fall of blood-pressure. Artificial respiratory movements slightly affected mechanically by the manipulation.

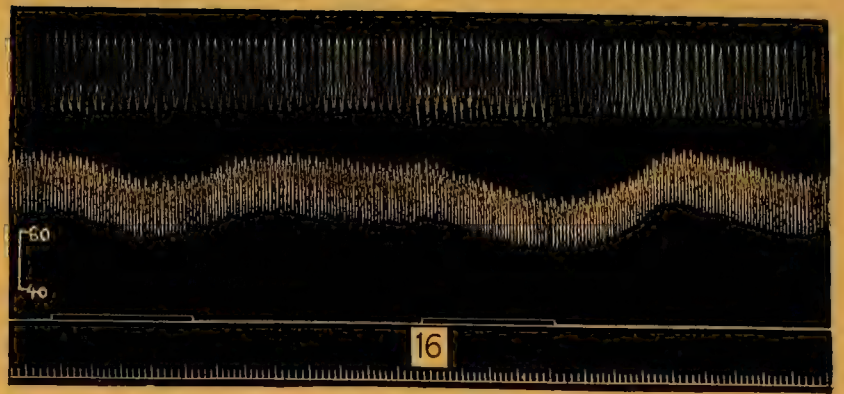
Fig. 18 Simultaneous tracings of the carotid blood-pressure and the volumes of kidney and hind limb. Dog. 10 kilos. 13/12/1918. Morphia and curare. Artificial respiration. Strong stimulation of the right sciatic nerve caused vascular constriction of the kidney, dilatation of the limb, and a rise of blood-pressure.

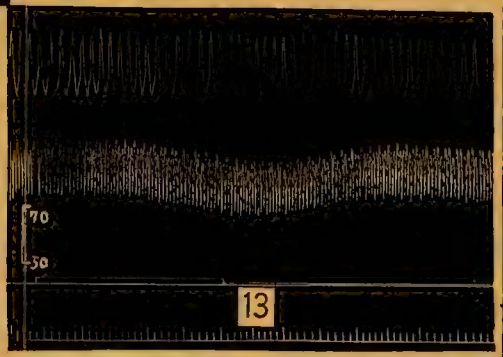
Fig. 19 Dog. 10 kilos. Ether. Effects of weak, moderate, and very strong stimulation of the skin. The weak stimulation gives a pure fall, the moderate stimulation a rise followed by a fall, while one very strong stimulation gives a pure rise.

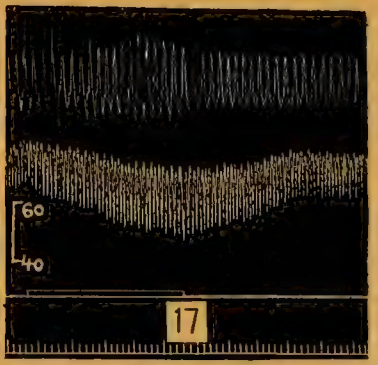

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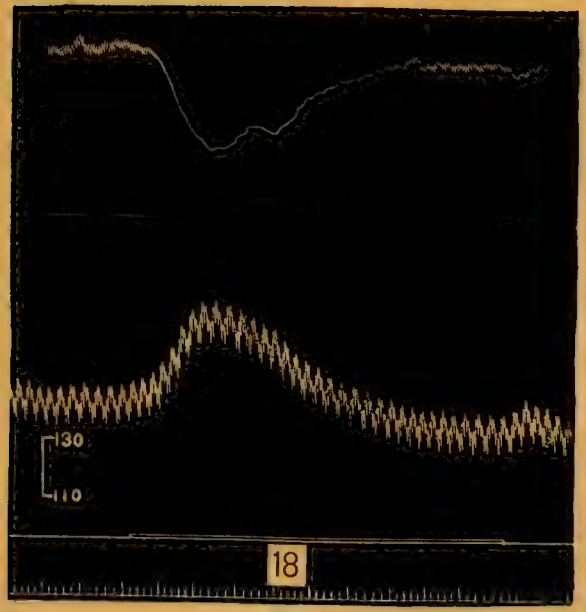

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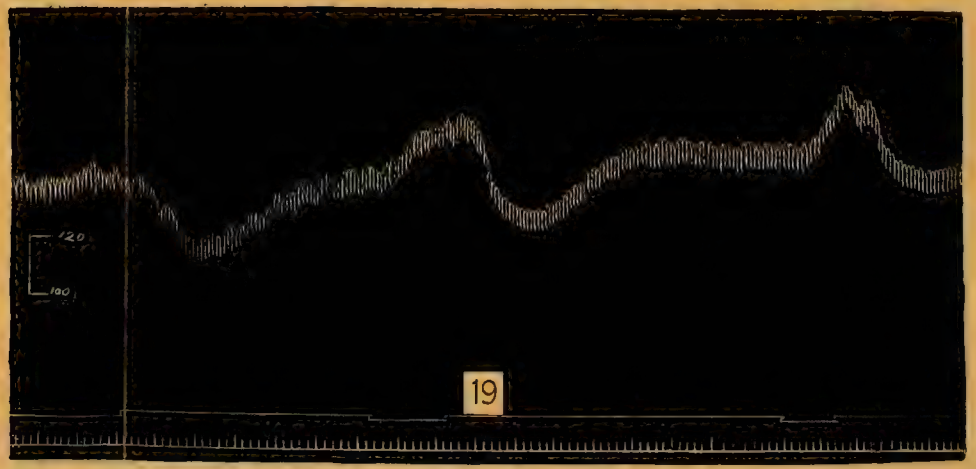

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JUNE 30

METABOLIC ACTIVITY OF THE NERVOUS SYSTEM

III. ON THE AMOUNT OF NON-PROTEIN NITROGEN IN THE BRAIN OF ALBINO RATS DURING TWENTY-FOUR HOURS AFTER FEEDING

SHIGEYUKI KOMINE

The Wistar Institute of Anatomy and Biology

THREE CHARTS

Hatai ('17) found in the nervous system of adult albino rats that the amount of non-protein nitrogen is surprisingly constant if the rats are examined under uniform nutritional conditions. It was thought interesting to determine whether or not there is a variation in the amount of non-protein nitrogen in the brain during the various stages of metabolism which occur in the course of the twenty-four hours following feeding. Should such variations be found, the study of them would throw some light on the very intricate problem of the metabolism of the nerve tissue.

As a first step I have undertaken to determine, therefore, the changes in the amount of non-protein nitrogen in the brain of the albino rat at intervals during the twenty-four hours which follow the last feeding, and the results so far obtained seem to me sufficiently interesting to publish.

MATERIAL

Albino rats were used for this study. The rats, which are usually fed at about 9 a.m., were removed after feeding into another cage, and kept without food, excepting water, until the following morning. The time during which the rats actually experienced lack of food is then approximately twenty-four hours. After twenty-four hours without food, the control rats were killed and examined, while those belonging to the group

to be tested received food, and after the required number of hours, were in turn examined for the non-protein nitrogen in the brain. It was found without exception that at the end of twenty-four hours without food the digestive tract is practically empty, although the lower part of the tract often contains some chyme.

In order to furnish a uniform diet for the test rats, we have always given them Uneeda biscuit mixed with condensed milk. Since some rats eat immediately, while others do not, we have placed enough food in the cage and left it there for exactly one hour, after which time the surplus food was entirely removed.

Our calculation of the time after feeding was started from the time when this surplus food was removed. We found the stomach always completely filled after one hour.

I have selected rats of more than 100 days old because Hatai ('17) states that the amount of non-protein nitrogen shows very slight age variation after the rats have passed this age. The younger the animal, the greater is the normal content of non-protein nitrogen in the brain in relation to its solids. The sexes were not distinguished in this investigation, but the control and test rats were taken from the same litter.

TECHNIQUE

The rats were killed with ether and the blood was removed by severing the carotid artery, followed by complete evisceration. The brain was removed as quickly as possible and the left half was used for the determination of the non-protein nitrogen, and the right for the water estimation. From the dried residue the total nitrogen was determined by the usual Kjeldahl method.

For the determination of the non-protein nitrogen I have followed the method adopted by Hatai ('17). According to this method, the brain was finely ground with 2.5 cc. of an aqueous solution of trichloracetic acid and then transferred to an Erlenmyer flask (50 cc.) with a small amount of distilled water. The amount of trichloracetic solution taken was always twenty times the brain weight in grams, which was expressed in volume, while the amount of water used was five times the brain weight

similarly expressed in volume. The mixture of tissue and reagents in the flask was shaken repeatedly during the first hour and then left for twenty-four hours at room temperature. The clear filtrate obtained from this extraction was now analyzed by Folin and Farmer's micro-method ('12) as modified by Benedict and Bock ('15). In all cases the nitrogen was estimated by means of the Duboscq colorimeter.

The water content of the brain was determined by drying it at 98°C. for one week and the total nitrogen by the usual Kjeldahl method.

It should be stated that as soon as the filtrates were completed the designation on each flask was replaced by a conventional mark put on by some other member of the laboratory, and thus the observer made the non-protein nitrogen determination in entire ignorance as to whether the specimen belonged to the control or to the test series. Thus no personal prejudice entered into the determinations, and since the difference between the controls and tests is not large, such a precaution was highly important.

THE NON-PROTEIN NITROGEN

Using forty controls and thirty-five test rats of both sexes, I have studied the amount of non-protein nitrogen in the brain during the twenty-four hours after feeding. The results together with several other incidental observations are recorded in table 1.

The number of milligrams of non-protein nitrogen per 100 grams of moist brain is evidently more uniform in the controls than in the test rats. This is of course to be expected if the non-protein content in the brain varies under different physiological conditions. However, when entire averages are taken, the values of the non-protein nitrogen given by both control and test rats are found to be identical. The average value of 157 mg. thus obtained unexpectedly coincides with the value found by Hatai in his determinations which were made on seventy-five brains of adult albino rats. This exact coincidence of the values is unquestionably accidental, but it shows that there is a sur-

TABLE 1

Showing the data on the amount of non-protein nitrogen of the brain, together with several other observations during twenty-four hours after feeding

Controls A

BODY WEIGHT	AGE	NUMBER RATS USED	BRAIN WEIGHT	WATER IN BRAIN	TOTAL NITROGEN IN BRAIN	NON-PROTEIN NITROGEN IN TOTAL NITROGEN	NON-PROTEIN NITROGEN PER 100 GRAMS BRAIN	DIFFERENCE
grams	*days*		*grams*	*per cent*	*per cent*		*mgms.*	
66.8	123	2	1.519	78.6	2.11	2.57	156	24
66.8	120	3	1.519	78.6	2.11	2.37	157	23
70.0	118	5	1.508	78.6	2.12	2.52	165	10
74.4	131	6	1.514	78.3	2.09	2.40	162	7
92.2	135	4	1.585	78.7	2.08	2.34	150	−11
96.3	135	6	1.590	77.9	2.09	2.33	148	3
98.6	112	3	1.573	78.7	2.13	2.36	152	−19
97.3	123	2	1.608	79.6	2.06	2.53	149	−21
99.6	161	3	1.611	78.4	2.11	2.57	163	−10
106.7	202	2	1.606	77.7	2.21	2.66	166	−5
131.6	173	2	1.670	79.2	2.22	2.20	135	−6
129.5	147	2	1.679	78.9	2.24	3.04	182	0
Average 94.5	140	40	1.582	78.6	2.13	2.49	157	

Tests B

NON-PROTEIN NITROGEN PER 100 GRAMS BRAIN	NON-PROTEIN NITROGEN IN TOTAL NITROGEN	TOTAL NITROGEN IN BRAIN	WATER IN BRAIN	BRAIN WEIGHT	NUMBER RATS USED	AGE	BODY WEIGHT	NUMBER OF HOURS AFTER FEEDING
mgms.		*per cent*		*grams*		*days*	*grams*	
180	2.88	2.16	78.4	1.590	2	123	89.1	2
180	2.84	2.22	79.0	1.533	3	120	85.9	3
175	2.72	2.21	79.6	1.548	5	118	91.5	4
169	2.64	2.16	78.4	1.533	5	131	83.0	5
139	2.25	2.13	78.8	1.623	3	135	93.5	6
151	2.40	2.16	79.0	1.590	4	135	109.5	7
133	2.24	2.08	78.0	1.675	2	112	144.4	8
128	2.31	2.15	78.6	1.549	2	123	121.6	9
153	2.48	2.19	78.1	1.615	3	161	133.1	10
161	1.70	2.23	77.9	1.701	2	202	137.8	11.5
129	2.32	2.26	78.8	1.590	3	173	133.8	16
182	3.03	2.24	78.3	1.668	2	147	116.7	20
Average 157	2.57	2.18	78.6	1.601	35	140	111.2	8.5

prising uniformity of non-protein nitrogen in the entire brain of the albino rat. Although the average values for the non-protein nitrogen content thus agree in both the control and test rats, yet the successive differences during the twenty-four hours after feeding are not at all similar, but the test rats show an interesting deviation from the controls. This deviation, or the difference between control and test rats, is well brought out by a graphic representation (chart 1).

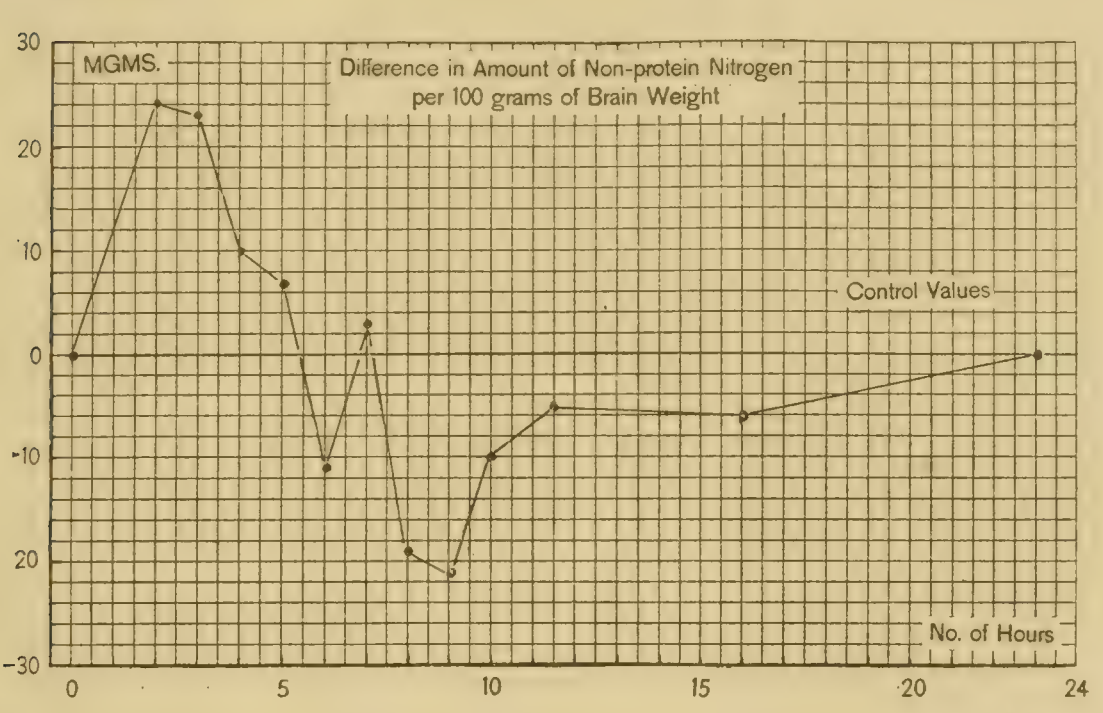


Chart 1 Showing the differences in the amount of non-protein nitrogen in the brain of test rats from that found in the controls.

From chart 1 we see clearly that the amount of non-protein nitrogen in the test animal increases very rapidly at first, and so far as the present data show, it reaches its maximum within two hours after feeding. This high content of non-protein nitrogen in the test brain soon begins to diminish, however, and within the next five or six hours the content reaches the original level found in the brain of the controls.

The diminution of the non-protein nitrogen in the test rats still continues steadily, and at eight or nine hours after feeding reaches a minimum, showing just as great a difference—in the

opposite direction—as was found between these two series at two hours after feeding. Soon after the content of non-protein nitrogen of the test brain has reached a minimum, at about eight or nine hours, the value begins to increase again, and finally at twenty-three hours after feeding the value for the non-protein nitrogen reaches once more the original level for the control rats.

The sort of variation just described was entirely unexpected, although an increase immediately after feeding and a diminution later were anticipated. The form of the graph at once indicates the periodic nature of the alteration in the content of non-protein nitrogen during the interval chosen. This periodic variation in the non-protein nitrogen content must of course be interpreted. It is evident that for a satisfactory understanding of this interesting phenomenon we must further analyze the non-protein nitrogen into its components, such as amino acids, urea, ammonia, creatinine, etc., as such a determination would reveal what sort of nitrogen was actually responsible for the variations shown by the graph. Although I have not as yet made an analysis of this kind, yet from the data given by numerous investigators, I feel justified in suggesting the following general interpretation.

The metabolic products from the digestive tract, which have been absorbed and then carried by the circulation, are reabsorbed from the blood by the brain tissue until this is saturated to a maximum degree with these metabolites.

In support of this view, there are abundant experimental data which show a quick absorption by the organs and tissues of non-protein substances injected into the circulation. For instance, Folin and Denis ('12) always obtained an increase in the non-protein nitrogen of muscle of the cat following the injection of amino acids, amino-acid digestion mixture, or Witte's peptone, into a ligated loop of intestine. Van Slyke and Meyer ('13–'14), by the now standard nitrous method, found an increase of amino acids in the muscles and several visceral organs of dogs after the injection into the venous blood of amino acids and protein digestion mixtures. Van Slyke and Meyer came to

the conclusion that the tissues rapidly absorb amino acids from the blood when their concentration in the fluid is increased.

The products thus absorbed in excess from the blood are probably utilized in part for rebuilding broken-down tissue, while at the same time the surplus which is not utilized, as well as the products formed by the catabolism of the brain tissue, are carried away by the circulation. This double process goes on in the brain until all the wear and tear is restored. The second period is represented by a marked reduction in the amount of non-protein nitrogen during the three to eight or nine hours after feeding. At the same time the amount of material to be absorbed from the intestine diminishes.

Here we encounter a difficulty arising from the fact that the amount of non-protein nitrogen in the test brains falls below that in the brain of the controls. It seems, however, probable that during the process of resynthetization of the polypeptides, some of the missing amino acids might be supplied by the amino acids that appear in the catabolic products constantly present in the brain tissue. The withdrawal of these amino acids from the normal content of metabolites in the brain tissue for utilization might be responsible in part at least for this smaller amount of non-protein nitrogen in the test brains at this period of active reconstruction.

This is, however, a pure hypothesis and therefore must await experimental examination.

When the minimum has been reached, and when no fresh supply of non-protein bodies is coming into the brain, catabolism becomes evident, and as a consequence, the amount of the metabolites again shows an increase. The slow rise from eight or nine hours up to twenty-four hours after feeding may represent this last period.

From the observations of Van Slyke and Meyer ('13–'14), that fasting increases the content of amino acids in the tissues and organs owing probably to autolysis, we may expect that the rise in the content of non-protein nitrogen after it has reached a minimum was also due to autolysis or to the phenomena of fasting.

Taking all these facts together, we conclude that after feed-

ing, the non-protein nitrogen content in the brain shows a definite periodic change. Under the same conditions, similar periodic phenomena should occur in other organs, and it will be highly interesting to test this inference on some other occasion. In this connection the observations made by Pepper and Austin ('15) on the content of non-protein nitrogen in the blood of dogs are very valuable. Pepper and Austin found that by feeding a dog with a moderate amount of meat, the blood nitrogen reaches a maximum within about two hours after feeding, and returns to the original level in about ten to fourteen hours.

When, however, the dog was allowed to fast, the blood nitrogen first falls gradually below the original level until it reaches a minimum at thirty to forty-eight hours, and then begins to rise gradually during a few hours, after which it tends to persist.

From the functions of the blood we can at once appreciate the relations of the observations of Pepper and Austin to the present study, because the blood receives the digestive products and distributes these to the tissues and organs.

When autolysis begins in the tissues and organs as the result of fasting, these autolytic products are again poured into the circulation. Consequently, what Pepper and Austin observed in the blood reveals what is probably happening in the organs and tissues. In reality the content of non-protein nitrogen in the blood indicates the periodic changes following first feeding and then fasting, as was observed by me in the brain of rats. Owing to the differences in the body size, as well as to the different degrees of activity of these two animals, the exact time relations found by Pepper and Austin cannot be directly compared with what has been found in the rat brain.

Very recently Mitchell ('18) studied the partition of non-protein nitrogen in the entire body, as well as in some organs of the albino rat at birth, before and after feeding. Mitchell noted in the younger rats a decided increase of non-protein nitrogen soon after feeding when compared with that in the rats fasting for twenty-four to forty-eight hours. In the case of adult rats, however, this increase was not as conspicuous as in the younger rats. The observed period following feeding was

not extensive enough (only up to seven hours after feeding), and thus a periodic variation in the non-protein nitrogen content, such as I have found in the brain, did not appear. Mitchell's observations, however, show clearly that the amount of amino acids and urea, as well as ammonia, are regularly higher in the fed than in the fasting animal, and furthermore, these fed rats show rapid increase in the non-protein nitrogen during the first five hours, and then slight decrease at seven hours, not only in the entire body, but in such organs as the kidneys, liver, and muscles. Despite the somewhat greater irregularities of the data given by Mitchell, the increase in the content of these simpler nitrogenous bodies during active protein digestion is well indicated.

RELATION OF THE NON-PROTEIN NITROGEN TO THE TOTAL NITROGEN

From the amount of non-protein nitrogen in the four divisions of the central nervous system of the rats, Hatai ('17) came to the following conclusions. "Although the amount of nitrogen given by the non-proteins—the amino acids, the urea and ammonia—in relation to the solids is higher in the cerebellum and cerebrum than either the stem or the spinal cord, these nitrogen values become constant in all the four parts when they are computed in relation to the protein nitrogen. This is interpreted to mean that the nitrogenous organic extractives are intimately related with the active cell substance, and not at all with the lipoid substance."

Following the statement of Hatai, it was thought desirable to find the relation which exists between the amount of non-protein nitrogen in the brain and the amount of total nitrogen, because by this means we can find a much closer relation between the active cell substance which is expressed in the present instance in terms of total nitrogen and metabolic products, owing to the elimination of myelin substance mainly.

The data for this determination are given in table 1, and I shall now discuss this observation as it appears in the graph given in chart 2.

It must be stated that in the total nitrogen those nitrogen values which belong to the non-protein substances, as well as those belonging to the lipoids, are also included. Any disturbance which may arise from such treatment should be very slight, owing to the greater amount of protein nitrogen contrasted with the nitrogen of the metabolic products. We find in chart 2 that in general the variation of non-protein nitrogen in relation to the total nitrogen is essentially similar to the relation found between entire brain substance and the non-protein nitrogen. This is to be expected, since the ages of rats examined are close to each other, and hence the water content, as well as the amount

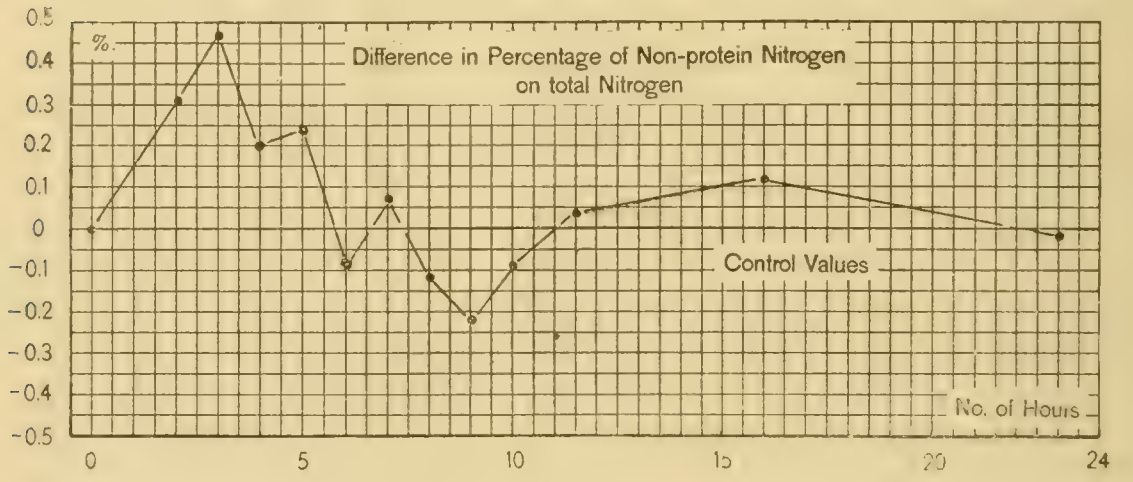


Chart 2 Illustrating the relation between the total nitrogen in the brain and metabolic products found during the twenty-four hours following feeding.

of myelin substance present in each brain, should not differ so much as they would, for instance, if the cerebrum was compared with the spinal cord of the same animal. This result was pleasing, since the close similarity found here, and that found in connection with the relation of the entire brain to the metabolites, shows the trustworthiness of the present technique which is suited to such experiments where very slight differences between the control and test animals are present.

We conclude from these additional facts that the non-protein nitrogen in the brain shows a periodic alteration during the twenty-four hours after feeding.

TABLE 2

Percentage of water in the brain

NUMBER OF HOURS AFTER FEEDING	OBSERVED			CALCULATED		
	Control	Test	Difference from control	Difference from control	Test	Control
2	78.6	78.4	−0.2	−0.1	78.1	78.2
3	78.6	79.0	−0.4	0.4	78.6	78.2
4	78.6	79.6	1.0	1.1	79.3	78.2
5	78.3	78.4	0.1	0.1	78.0	77.9
6	78.7	78.8	0.1	0.1	78.5	78.4
7	77.9	79.0	1.1	1.1	78.7	77.6
8	78.7	78.0	−0.7	−0.7	77.6	78.3
9	79.6	78.6	−1.0	−1.1	78.2	79.3
10	78.4	78.1	−0.3	−0.3	77.8	78.1
11	77.7	77.9	0.2	0.4	77.7	77.3
16	79.2	78.8	−0.4	−0.5	78.4	78.9
23	78.9	78.3	−0.6	−0.6	78.1	78.7
Average 8.5	78.6	78.6	−0.3	−0.1	78.3	78.3

PERCENTAGE OF WATER IN THE BRAIN

It is conceivable that following the greater or less accumulation of the metabolic products in the brain, the water content might show a change also. We have determined the amount of water in the brain of the control and test rats and found that while their average observed values are identical, both being 78.6 per cent (tables 1 and 2, 'Observed'), yet their successive differences during the twenty-three hours after starvation are not identical, but show interesting deviations. In dealing with the values on the percentage of water, we must consider, in the interest of precision, the influence of brain size on the water content.

Donaldson ('16) finds that although the percentage of water in the brain is a function of age, nevertheless, within the same age, the heavier brain gives relatively less water than the lighter brain, and vice versa.

Since, then, the brain weights given by both the control and test rats are not identical, we thought it advisable to transform the observed values of the percentage of water to the theoretical

values in which the influence of the difference in brain weight is entirely eliminated. The exact method of correction is given by Donaldson ('16) and for it the reader is referred to that paper. The percentage of water in each instance, as thus calculated, is also given in table 2. We notice that the differences obtained when the calculated values are used are practically identical with those obtained by the use of the observed values, but since the observed brain weights were small for their age, the absolute observed values for the percentage of water in both control and test rats run above those which are calculated. We have, however, chosen the calculated values of the percentage of water for the construction of chart 3.

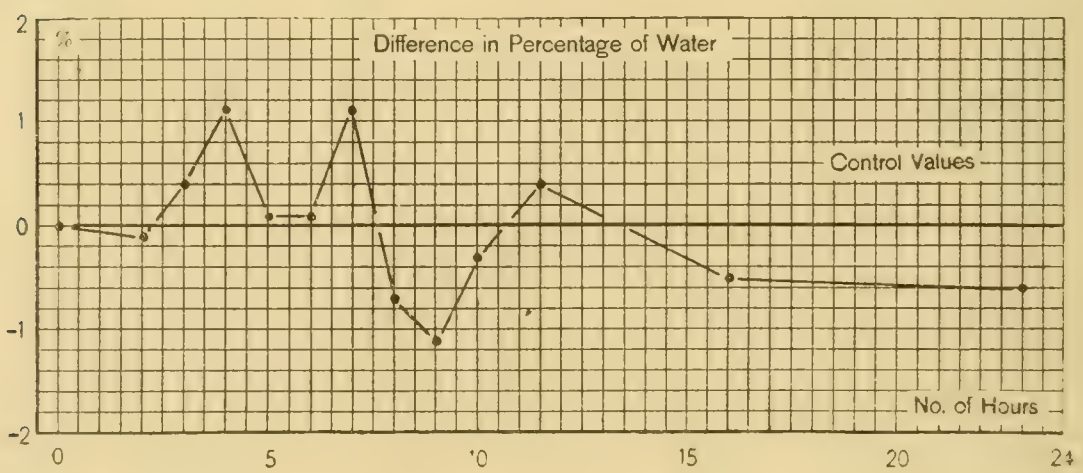


Chart 3 Showing the differences in the calculated water content of the brains of the test rats compared with those of the control rats.

It is evident at once that the relative water content in the brain of the test rats is definitely higher during the first seven hours, and then falls below that of the controls and reaches its minimum at about nine hours, and so far as the present data are concerned, the relative water content of the test brains again rises, tending at the end of the period to approach the original level. On account of the irregularities, it is not desirable to place too much stress on the exact form of this graph; nevertheless, it gives unquestionable evidence of an increase in the water content during the first seven hours, followed first by a sharp fall and then by a return toward the normal or control value.

It is also evident that this variation in the water content as the result of feeding is in general similar to the variation which is shown by the content of non-protein nitrogen in the brain, although the exact time relations of the rise or fall of the curves are not identical.

From these results I am inclined to think that the percentage of water in the brain rises with the increase in the non-protein nitrogen and falls with its decrease, but this is merely a tentative conclusion which must await more careful study.

CONCLUSIONS

1. During the twenty-four hours after feeding the increase of non-protein nitrogen in the rat's brain reaches its maximum at from two to three hours after the taking of food. This rapid rise is followed by a steady diminution which reaches a minimum at about eight or nine hours. The non-protein nitrogen then shows a steady but slow increase and reaches the original level at about twenty-three hours. In other words, non-protein nitrogen in the brain shows a periodic alteration and completes one period within about twenty-four hours after feeding.

2. This periodic change depends on the ingestion of food. Non-protein nitrogen appears in the brain as the result of the absorption of such material from the digestive tract and the formation of such bodies by the catabolic activity of the brain tissue itself. Its amount is diminished by the anabolic process in the brain tissue and by excretion. The variation of its amount in the brain is a resultant of these several processes.

3. A similar periodic relation is shown when the non-protein nitrogen is compared with the total nitrogen instead of the entire brain mass.

4. The course of the percentage of water in the brain follows that of the non-protein nitrogen.

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Resumen por el autor, James Stuart Plant.
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Factores que influyen en el comportamiento del cerebro de la rata albina en el liquido de Müller.

El cerebro de la rata albina sufre un cambio típico cuando se "fija" en el liquido de Müller, compuesto de 2 por ciento de bicromato potásico y 10 por ciento de sulfato sódico. El cerebro aumenta rápidamente de peso, al que sigue una pérdida de peso lenta y continua, hasta que al cabo de setenta y cinco dias, al pesar el cerebro se comprueba que pesa de 20 a 30 por ciento más que el cerebro fresco. 1. La edad es la principal condición que influye en esta reacción del liquido de Müller. Los cerebros de ratas viejas aumentan más en peso y retienen este aumento durante los setenta y cinco dias. 2. El peso inicial del cerebro, o sea su tamaño, es la condición de más importancia después de la apuntada más arriba. Del mismo modo que con los cerebros de la misma edad, los cerebros más ligeros aumentan más de peso durante la primera parte de su permanencia en el liquido de Müller. Esta diferencia disminuye gradualmente y desaparece al cabo de los setenta y cinco dias. 3. La edad próximamente semejante (entre ciertos límites) tiene casi el mismo valor determinante que la igualdad de edad. 4. El sexo es un factor sin importancia, del mismo modo que la composición hereditaria (relación dentro de un tronco determinado).

Translation by José F. Nonidez
Carnegie Institution of Washington

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JUNE 30

FACTORS INFLUENCING THE BEHAVIOR OF THE BRAIN OF THE ALBINO RAT IN MÜLLER'S FLUID

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The brain of the albino rat, placed for a period in Müller's fluid, exhibits a typical change. In the course of time it not only hardens, but also markedly increases in weight. There is a rapid increase to a maximum in about one week's time, after which there is a slow, steady loss until the seventy-five day weighing, at which time the brain weighs 20 to 30 per cent more than when fresh. It was thought that changes in this typical curve might be induced in the brains of rats previously anesthetized for prolonged periods, and it was hoped that this criterion would be more delicate than the microscopic or analytic tests which had, so far, failed to demonstrate a change. The work was done at The Wistar Institute of Anatomy during the academic year 1913–1914.

PLAN

The main question of the effect of the anesthetic on the typical curve remains unanswered. From the start, however, it was recognized that various factors influenced the reaction of 'control' brains to Müller's fluid—factors which are inherent in the material. It is these factors and their influence on the reaction with which the present paper deals.

PROCEDURE

The brains studied belonged to 'stock' albino rats. The animals were killed with ether and the brains quickly removed with every care not to damage them. They were immediately weighed and then suspended in 50 cc. of Müller's fluid. The

whole was kept in a black cardboard case in a dark closet. Subsequent weighings were as follows. The brain was removed from the solution and placed for about ten seconds on a dry piece of filter-paper. The string by which it had been suspended was during this time removed. The brain was then placed on a watch-glass and immediately weighed. It was returned to the Müller's fluid as quickly as possible. The watch-glass was then weighed. Reweighings were carried out at 24 hours, and at 7, 14, 30, and 75 days after killing the rat. On completion of the weighings the percentage of water in the brain at the final weighing was determined. This procedure involved placing the brain, immediately after its last weighing, in a small glass vial of known weight. This was kept in a drying oven (temperature, 97°) for one week. On removal, the vial was cooled in a desiccator at room temperature and weighed.

The Müller's fluid used was made up in 1000 cc. lots. To 25 grams of potassium bichromate c. p. and 10 grams of sodium sulphate c. p. was added 1000 cc. of distilled water. Time was given for dissolving the salts and, after thorough agitation, the solution was divided into two 500 cc. lots and kept for one month before being used. In every instance 'pairs' of brains were fixed in fluid from the same bottle. The Müller's fluid was always kept in a dark closet.

No attempt was made to control the temperature during the reaction of fixation other than that all specimens were kept in the same dark closet at room temperature. Thus the results may be considered as comparable.

Necessarily our original results are in terms of absolute weights and absolute gains. In the presence of so diverse initial weights it seemed, however, best to state all gains in weight as percentages of the original weight. This makes the data comparable. Corresponding to this, all statements of the relation of one brain's gain to that of another are in terms of a percentage of the percentages of gain of the heavier brain. This leads to higher figures, in the relations of the gains, than would be the case were the actual differences between the absolute gains stated.

OBSERVATIONS

If we consider the brains of pairs (rats of the same age, sex, and litter, i.e., as similar as possible), there appears a very distinct tendency for the brains of older rats to gain more in Müller's fluid than do those of the younger rats. Table 1 presents fifteen pairs arranged on the basis of their age. In ten of the fourteen possible comparisons the brains of older rats gain more in twenty-four hours. Also in ten of the comparisons the older brains

TABLE 1

Percentages of gain of pairs of albino rat brains arranged according to age and weighed at intervals from twenty-four hours to seventy-five days

SEX	AGE	INITIAL WEIGHT		AVERAGE PERCENTAGES OF GAIN OF BOTH BRAINS IN MÜLLERS FLUID				
		1	2	24 hours	7 days	14 days	30 days	75 days
	days	*grams*	*grams*					
♀	52	1.465	1.368	19.0	28.2	26.2	23.5	22.9
♀	55	1.615	1.578	20.8	32.1	28.1	26.8	25.4
♂	57	1.757	1.671	20.6	31.2	26.0	25.9	25.7
♂	59	1.635	1.519	21.2	33.1	29.9	27.0	26.7
♂	61	1.834	1.715	18.0	32.2	28.0	25.9	25.0
♀	61	1.699	1.581	20.0	29.5	27.3	24.1	23.0
♂	62	1.490	1.477	18.6	31.8	28.3	25.3	24.4
♂	62	1.662	1.656	19.3	28.2	24.6	23.1	22.7
♀	62	1.497	1.496	20.5	31.7	29.1	25.7	25.3
♂	62	1.831	1.699	20.8	32.8	30.8	28.0	27.8
♀	64	1.677	1.606	20.9	32.0	28.6	26.2	25.7
♂	67	1.651	1.587	21.7	32.6	29.7	26.6	26.4
♀	72	1.610	1.492	27.6	33.6	30.7	27.9	26.7
♂	160	1.791	1.752	25.1	37.2	35.8	32.8	32.2
♂	218	2.008	1.824	28.3	40.0	38.6	35.7	34.6

gain more in seventy-five days, though this does not in every case involve the same comparisons as were favorable to the older rats at the twenty-four hour weighing. Table 2 presents a summary of the data of table 1. The averaged figures for the youngest three pairs and for the oldest three pairs are given. The data show that age is a very important factor in the reaction of the brain of the albino rat to Müller's fluid.

Brain weight increases as a function of age, and there exists between these two characters a very high coefficient of correla-

TABLE 2

Percentages of gain of averaged entries of table 1

PAIRS AVERAGED	AGE	INITIAL WEIGHT		AVERAGE PERCENTAGES OF GAIN IN MÜLLERS FLUID				
		1	2	24 hours	7 days	14 days	30 days	75 days
	days	*grams*	*grams*					
First three entries.....	55	1.612	1.539	20.1	30.5	26.8	25.4	24.7
Last three entries.....	150	1.803	1.689	27.0	37.0	35.0	32.1	31.2

tion. If we make a comparison of the individual brains of the respective pairs involved in table 1, however, we may study the effect of initial brain weight in animals of like age. The data are given in table 3. In place of the percentage of gain of the lighter brain there is entered at the several columns marked 'Per cent deviation of 2'—under 'Time in Müller's fluid'—only the relation of that percentage to the percentage of gain of the heavier brain. Of the fifteen pairs involved, it will be noted that in twelve the lighter brain gains more in the first twenty-four hours (represented by a + in the second column). Also in twelve of the fifteen pairs, the heavier brain later 'catches up' —that is, the relative gain of the heavier brain is greater at seventy-five days than at one day. This phenomenon is clearly demonstrated in the 'averages' at the bottom of the table. The results may be summarized as follows:

	1 DAY	7 DAYS	14 DAYS	30 DAYS	75 DAYS
Average difference.....	+3.9	+1.5	+1.3	+1.2	+0.2
Standard deviation.....	±6.1	±3.9	±5.0	±5.2	±6.5

Thus it appears that the lighter brain gains more in the early part of the stay in Müller's fluid, but that this difference practically disappears at the seventy-five-day weighing. It is to be noted that the standard deviations are lowest at the seven-day weighing. This seems to represent the period of least individual variation. As this represents the time of maximum increase, we may consider that as the more stable period in the

curves and think of the rise and fall in percentage of increase as periods more subject to individual variation.

While increasing age presupposes increase in brain weight, it is apparent that these two—age and brain weight—act as opposing factors in the determination of the reaction of the brain to Müller's fluid. That is, the older brains (these are heavier)

TABLE 3

The effect of initial brain weight—albino rat—on the percentage of gain of paired brains. Pairs arranged according to age. Deviation of the lighter brain

SEX	AGE	INITIAL WEIGHT		TIME IN MÜLLERS FLUID. PERCENTAGES OF GAIN									
				24 hours		7 days		14 days		30 days		75 days	
		1	2	1	Per cent deviation of 2	1	Per cent deviation of 2	1	Per cent deviation of 2	1	Per cent deviation of 2	1	Per cent deviation of 2
	days	*grams*	*grams*										
♀	52	1.465	1.368	19.3	− 2.8	27.9	+2.1	26.8	−4.2	24.1	− 4.9	23.5	− 5.7
♀	55	1.615	1.578	20.4	+ 3.6	31.7	+2.5	27.7	+2.6	25.8	+ 8.2	25.1	+ 2.1
♂	57	1.757	1.671	19.5	+11.1	30.5	+4.1	25.5	+4.1	25.2	+ 5.1	24.8	+ 6.8
♂	59	1.635	1.519	20.5	+ 6.5	33.0	+0.2	30.3	−2.2	27.4	− 2.4	27.2	− 4.0
♀	61	1.699	1.581	18.8	+13.3	28.7	+6.0	26.2	+8.8	23.3	+ 7.0	21.6	+12.9
♂	61	1.834	1.715	17.9	+ 1.3	31.5	+4.2	27.5	+4.1	25.4	+ 3.8	24.7	+ 1.8
♂	62	1.490	1.477	18.8	− 1.8	32.4	−3.4	29.3	−7.2	26.3	− 7.3	25.0	− 4.8
♀	62	1.497	1.496	20.5	+ 0.3	31.4	+1.7	28.7	+2.1	25.9	− 0.7	25.1	+ 2.1
♂	62	1.662	1.656	19.2	+ 0.9	27.4	+6.0	23.7	+7.2	22.6	+ 4.4	22.2	+ 4.5
♂	62	1.831	1.699	18.9	+19.6	31.5	+8.4	29.4	+9.8	26.7	+10.3	26.3	+11.4
♀	64	1.677	1.606	21.1	− 1.2	32.6	−3.5	29.4	−4.9	26.8	− 4.3	26.2	− 4.3
♂	67	1.651	1.587	21.6	+ 0.8	33.3	−4.6	30.5	−5.1	27.3	− 4.7	28.0	−11.2
♀	72	1.610	1.492	26.9	+ 5.2	33.3	+1.3	30.4	+1.7	28.3	− 2.3	27.4	− 5.2
♂	160	1.791	1.752	25.0	+ 0.3	38.0	−4.0	35.3	+2.4	32.6	+ 1.1	32.5	− 2.2
♂	218	2.008	1.824	28.0	+ 2.0	40.1	+1.2	38.5	+0.9	34.8	+ 4.9	34.7	− 0.8
Average					+ 3.9		+1.5		+1.3		+ 1.2		+ 0.2
Standard deviation					± 6.1		±3.9		±5.0		± 5.2		± 6.5

gain more than do the younger ones; yet the lighter brains gain more than do the heavier ones if we can eliminate age as a factor, as was done in table 3. The curve of increase in weight may be considered as capable of solution into at least two curves —expressing these two factors. Age appears to be by far the more potent factor.

A further study was made of fifty-nine brains arranged according to increasing brain weight but without regard to sex or litter. In table 4 these are arranged according to initial brain weight in three age groups. Within these groups two phenomena are apparent (shown in the averages under the vertical column, 'Percentage difference from the following value'). These are the early greater gains for the lighter brains (this does not hold clearly for the ten brains of the youngest group where there is practically no difference); and the fact that at the seventy-five-day weighing the lighter brains show relatively a less percentage of increase than they do at the twenty-four-hour weighing. Since these facts are just those which determine the curve when brains of rats of the same age, sex, and litter are compared, we may conclude that in the reaction of the brain to Müller's fluid:

1. Sex is negligible.
2. Inherited composition is negligible.
3. Approximate similarity of ages (the range being limited) may be considered as having the same effect as though the ages were identical.

The data on the percentage of water—in the last column of table 4—will be discussed later.

A group of four brains—all belonging to young rats—was subjected to an additional procedure. The brains, immediately upon removal, were separated into cerebrum, cerebellum, stem, and olfactory bulbs. Each part was then treated as were the whole brains of the other series. The data are given in table 5 in this way, that that percentage of the whole brain weight represented by the weight of each part at each weighing is recorded. The figures for the four brains show but slight variation, and table 5 therefore presents only the averages of the four. The relative weights of the various parts undergo considerable change in Müller's fluid, but this change is mainly consummated in the first twenty-four hours. Thus we may assume from this study that, while the relations of the various parts are altered in the fixing solution, the length of time, after the first twenty-four hours, during which the parts are subjected to this treatment, is a matter of minor import.

TABLE 4

The effect of initial brain weight—albino rat—on the percentages of gain of brains arranged in age groups, regardless of sex or litter

INITIAL WEIGHT (GRAMS)	NUMBER OF CASES	AVERAGE AGE	24 HOURS	PERCENTAGE DIFFERENCE FROM FOLLOWING VALUE*	AVERAGE PERCENTAGES OF GAIN					
					7 days	14 days	30 days	75 days	Percentage difference from following values.	Per cent of water at 75 days
50 to 60 days										
1.35–1.40	1	52	18.7	− 2.8	28.5	25.7	22.9	22.2	− 5.6	79.3
1.40–1.50	1	52	19.3	−11.1	27.9	26.8	24.1	23.5	−11.2	79.3
1.50–1.60	3	53	21.7	+ 5.8	33.2	29.2	27.5	26.5	+ 1.2	80.1
1.60–1.65	2	57	20.5	− 2.2	32.3	29.0	26.6	26.2	− 3.0	79.9
1.65–1.70	2	58	20.9	+ 3.2	32.8	28.8	27.7	27.0	+ 8.7	80.3
1.70–1.80	1	57	19.5		30.5	25.5	25.2	24.8		80.3
Average.1.58	10	55	20.5	− 0.9	31.7	28.1	26.3	25.7	− 2.1	80.0
60 to 70 days										
1.40–1.50	7	64	21.7	+ 1.9	32.9	29.8	26.6	25.7	+ 0.5	79.7
1.50–1.60	5	65	21.3	− 8.6	32.1	29.1	26.2	25.6	− 9.3	79.3
1.60–1.65	6	69	23.3	+12.3	34.1	31.5	28.8	28.2	+10.6	80.2
1.65–1.70	11	68	20.7	+ 2.9	31.5	28.5	26.0	25.5	+ 3.1	79.7
1.70–1.80	5	65	20.1	+ 8.2	31.6	28.2	26.4	24.7	− 1.8	80.1
1.80–1.90	3	63	18.6		31.5	27.9	25.7	25.2		80.7
Average.1.65	37	66	21.1	+ 3.9	32.3	29.2	26.6	25.9	+ 1.8	79.8
180 to 240 days										
1.65–1.70	1	189	28.7	+ 8.5	42.4	39.2	36.1	35.4	+ 9.3	81.6
1.70–1.80	4	191	26.5	+ 2.8	38.2	36.2	33.2	32.4	+ 3.2	80.5
1.80–1.90	5	239	25.7	+ 8.7	37.6	36.4	32.4	31.5	+ 6.1	80.5
1.90–2.00	2	206	23.7		35.6	33.0	30.4	29.7		78.4
Average.1.83	12	213	25.9	+ 6.1	37.9	36.0	32.7	31.8	+ 5.6	80.2

* The percentages were obtained originally by the use of values carried to three places. These have now been reduced to one-place numbers and there are therefore some apparent discrepancies in the percentage-difference columns. These differences, however, are not significant.

TABLE 5

Averaged percentage weight relations of the parts of four albino rat brains during the course of their reaction to Müller's fluid

AGE	WEIGHT OF WHOLE BRAIN AVERAGE OF FOUR	PERCENTAGE REPRESENTED BY CEREBRUM	PERCENTAGE REPRESENTED BY STEM	PERCENTAGE REPRESENTED BY CEREBELLUM	PERCENTAGE REPRESENTED BY OLFACTORY BULBS	TIME IN MÜLLER'S FLUID
52	1.610	65.17	17.40	13.80	3.63	Initial
	2.127	63.10	17.68	15.08	4.14	24 hours
	2.171	64.82	17.21	14.33	3.63	7 days
	2.094	63.77	17.76	14.72	3.75	30 days
Difference between percentages at initial and 24-hour weighing.....		−2.07	+0.28	+1.28	+0.51	
Difference between percentages at 24-hour and 30-day weighing....		+0.67	+0.08	−0.36	−0.39	

WATER RELATIONS

We have studied the water relations after seventy-five days in fifty-nine whole brains and after thirty days in the parts of three brains. There is evidently, in the reaction of the brain to the Müller's fluid, a deposition of salts in the brain tissue. This is shown in table 6. Part A deals with the fifty-nine whole brains; Part B with the parts of three brains (all belonging to young females). The deposition of salts at the end of seventy-five days in the whole brain is from 3.9 per cent to 4.3 per cent of the total water of the brain despite the fact that the salts in Müller's fluid are present in a concentration of but 3.5 per cent. This shows a deposition of salts in the tissues. If, in addition, there is some diffusion of solids from the brain to Müller's fluid—and, from inspection, this appears to be the case—the percentage of salts deposited must be even higher than that indicated by the figures given.

The final percentage of water in a given brain at the seventy-five-day weighing is only slightly greater than that of a fresh brain belonging to a rat of the same age, sex, and litter. In view of the 20 to 30 per cent net increase in weight in Müller's

TABLE 6

Part A. Water relations at the seventy-five day weighing of fifty-nine whole brains (see table 4)

	UNDER 60 DAYS	60 TO 120 DAYS	OVER 120 DAYS
Average fresh brain weight	1.582	1.651	1.826
Percentage of water (from Donaldson, '16)	79.1%	78.9%	78.1%
Calculated amount of water represented in fresh brain	1.252	1.303	1.426
Final brain weight	1.988	2.078	2.407
Final amount of water	1.590	1.659	1.932
Percentage of water—observed	80.0%	79.8%	80.2%
Increase in weight due to water, gms	0.338	0.356	0.506
Increase in weight due to salts, gms	0.068	0.071	0.075
Percentage of salts in total increase in weight	16.6%	16.7%	13.0%
Percentage of salts in the total water in brain	4.2%	4.3%	3.9%

Part B. Water relations at the thirty-day weighing of parts of three brains

	CEREBRUM	STEM	CEREBELLUM	OLFACTORY BULBS
Average fresh brain weight	1.097	0.292	0.235	0.058
Percentage of water (from Donaldson, '16)	80.0%	76.1%	79.7%	82.3%
Calculated amount of water represented in fresh weight	0.878	0.222	0.188	0.048
Final weight	1.403	0.386	0.329	0.078
Final amount of water	1.139	0.301	0.266	0.067
Percentage of water—observed	81.2%	77.9%	80.8%	85.6%
Increase in weight due to water, gms	0.261	0.079	0.078	0.019
Increase in weight due to salts, gms	0.044	0.016	0.015	0.001
Percentage of salts in total increase in weight (for total brain 14.8%)	14.5%	16.4%	16.4%	4.9%
Percentage of salts in total water in the part (for total brain 4.3%)	3.9%	5.0%	5.8%	1.5%

fluid, this seems a striking fact, though where the initial percentage of water is so high, it is evident that it takes a relatively large difference in the absolute water content to markedly affect the percentage value.

In the three brains divided into their parts the salts are deposited in the following percentages after thirty days:

Cerebellum	5.8 per cent
Stem	5.0 per cent
Cerebrum	3.9 per cent
Olfactory bulbs	1.5 per cent
Average of whole brain	4.3 per cent

With the exception of the cerebellum, the percentage of salts deposited is in direct relation with the proportion of myelin in the part involved. As none of the parts were washed, it may well be that the interstices of the cerebellum were the site of large deposits of salts, a physical factor which may account for this anomalous result.

CONCLUSIONS

General reaction of the brain to Müller's fluid, or type curves. The brain of the albino rat undergoes a typical change when 'fixed' in Müller's fluid (2.5 per cent potassium bichromate and 1 per cent sodium sulphate). There is a rapid increase in weight followed by a slow, steady loss until at the seventy-five-day weighing the brain weighs 20 to 30 per cent more than when fresh.

Factors affecting this reaction, or components of the type curve.

1. Age is the main condition controlling this reaction to Müller's fluid. The brains of older rats gain more and retain this higher relative gain throughout the seventy-five days.

2. Initial brain weight, or size, is the condition of next importance. As between brains of like age the lighter brains gain more during the earlier part of their stay in Müller's fluid. This difference is gradually lessened, and it disappears at the seventy-five-day weighing.

Thus, while age and initial brain weight are highly correlated, they constitute factors which, when taken alone, influence in opposite ways the reaction of the brain to Müller's fluid. The early greater increase of the smaller brain of two rats of the same age may be due to one or both of the following factors:

a. If we consider the brain as a sphere and the fluids as penetrating at a fixed rate, then in the smaller brain a slightly greater

proportion of the brain will be penetrated—that is, swollen by the fixing fluid—at any given instant in the early part of the reaction. This would give a more rapid enlargement in the smaller brain.

b. The smaller brain has a higher percentage of water which might make diffusion more rapid. If this is a controlling factor, the matter of a higher percentage of water must be of more immediate importance than is that of a lesser percentage of myelin.

3. Approximately similar age (the range being limited) has nearly the same determining value as equality of age.

4. Sex is a negligible factor, as is also inherited composition (relationship within a given strain).

FINAL WATER RELATIONS

The increase in weight is due mainly to the taking up of water, but the percentage of salts deposited in the fixed tissues is much greater than that in the fixing fluid. With the exception of the cerebellum, the deposition of salts is proportional to the myelin present in the part of the brain.

RELATION OF RESULTS

The curve of reaction of 'control' brains to Müller's fluid is evidently of such constancy in character as to make it a satisfactory criterion for a judgment as to alterations produced in the brain by various experimental procedures. It seems that in problems involving changes not available to such microscopic or analytical tests as we have, this reaction might furnish a valuable means of study. It appears from the foregoing results that when tests are made for the experimental modification of the response of the brain to Müller's fluid, it is necessary to have the test and control brains of the same age and, in those cases in which the brains differ in weight, to allow sufficient time for the compensation of this difference.

LITERATURE

While various observations bearing upon this problem have been made, none have employed exactly our experimental procedure. Various solutions of potassium bichromate have been used—Donaldson ('94), Fish ('93), and King ('10)—but a study in the changes in the reaction to Müller's fluid has not been made. A similar study was carried out by Hrdlicka ('06) in which various formalin preparations were used as fixing reagents. The effect of age upon this reaction has been discussed in a general way—Donaldson ('94) and King ('10)—but it seems that the extent of its control over the reaction has not been previously stated.

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Resumen por el autor, Olof Larsell.
Universidad de Wisconsin.

Estudios sobre el nervio terminal: la tortuga.

El autor describe las relaciones periféricas y la distribución del nervio terminal en el embrión de la tortuga. Un plexo del nervio, situado en el tabique nasal, está intimamente mezclado con un plexo de la rama oftálmica del nervio trigémino. El autor compara las células ganglionares del nervio terminal, en lo referente al tamaño y caracteres morfológicos generales, con las células de los ganglios sensorio y del simpático de los mismos embriones. Las células del nervio terminal presentan una semejanza sorprendente, tanto en el tamaño como en la forma, con las células halladas en los ganglios del simpático, especialmente las de los situados en la región cefálica. Las células de los racimos ganglionares del nervio terminal no pueden diferenciarse de células emigrantes que se presentan a lo largo de la rama oftálmica del trigémino. Todo esto indica que el nervio terminal está relacionado con el sistema del simpático.

Translation by José F. Nonidez
Carnegie Institution of Washington

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JUNE 30

STUDIES ON THE NERVUS TERMINALIS: TURTLE[1]

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SIXTEEN FIGURES

The present contribution was begun as part of a comparative study of the nervus terminalis in several groups of vertebrates. The work had not progressed far before it was found advisable to confine attention to one group at a time, so that the greater portion of the present report embraces the results of observations made since the studies on the nerve in mammals by the author ('18) was published.

The somewhat extensive literature of the nervus terminalis was reviewed in the previous article, and only two papers which have appeared on the subject during the past year will be mentioned briefly. These papers are by Van Wijhe ('18) and Ayers ('19).

Van Wijhe's paper reviews much of the literature of the nervus terminalis in the various groups of vertebrates briefly and homologizes the nerve with one he noted a number of years ago ('94) in Amphioxus, which he termed at that time the 'nervus apicis.' He states: "Before the homologue of the profound ophthalmicus there is in Amphioxus still another nerve which supplies the utmost point of the snout. On account of this and because it arises from the morphological fore-end of the cerebral ventricle I called it the nervus apicis."

Ayers ('19), in continuing his studies of Cephalogenesis, begun long ago, has found the nervus terminalis (Van Wijhe's 'nervus apicis') in Amphioxus, and calls attention to its large size as

[1] Contribution from the Zoological Laboratory of Northwestern University, William A. Locy, Director, and from the Anatomical Laboratory, University of Wisconsin.

compared with the olfactory nerve in that form. He finds it also in Cyclostomes and states that in Bdellostoma the nerve presents an intermediate stage, as respects its size and relations, between Amphioxus and the selachians. He calls attention to the distinction between the vomeronasal nerve, which he terms the 'nervus septalis' and the olfactory nerve, and suggests a new classification of the cranial nerves in which the nervus terminalis would be number I, and, with the 'nervus septalis', would be added to the list of twelve cranial nerves usually recognized. The nervus terminalis is considered a sensory nerve which has to do with a group of chemical sense organs, and is related physiologically to the vomeronasal (his septal) nerve. The conclusions are reached from a study of Amphioxus and cyclostomes. Doctor Ayers believes that in higher forms the nerve has undergone considerable modification due to changes in head structure.

I am under great obligation to Doctor Ayers for opportunity to read his manuscript prior to publication and for permission to make use of his observations. He also afforded me opportunity to read Van Wijhe's paper, which I had not previously seen. It is a pleasure to express here my sense of indebtedness to him. My acknowledgments are also due Prof. William A. Locy, of Northwestern University, under whose direction the general problem was originally begun and who has since continued his interest.

MATERIAL AND METHODS

Embryos of the painted turtle (Chrysemys marginata) were used. Most of these had been fixed in a formol-bichromate-acetic fluid, some in formalin of 10 per cent, others in Tellyesniczky's fluid, and a few living embryos were obtained and prepared by the Cajal and the Vom Rath methods.

Stages beyond 10- to 11-mm. carapace length had become chitinized to such an extent in the rostral region that intact serial sections could not be obtained. Chiefly for this reason, the present contribution is confined to a description of the nervus terminalis in embryos up to 11-mm. carapace length (about 17 mm. greatest length.

Numerous dissections of embryos and of newly hatched turtles were made with the aid of the binocular microscope. The head was split slightly to one side of the midsagittal plane, and the soft parts were then sufficiently removed to expose the nerve and its adjacent structures.

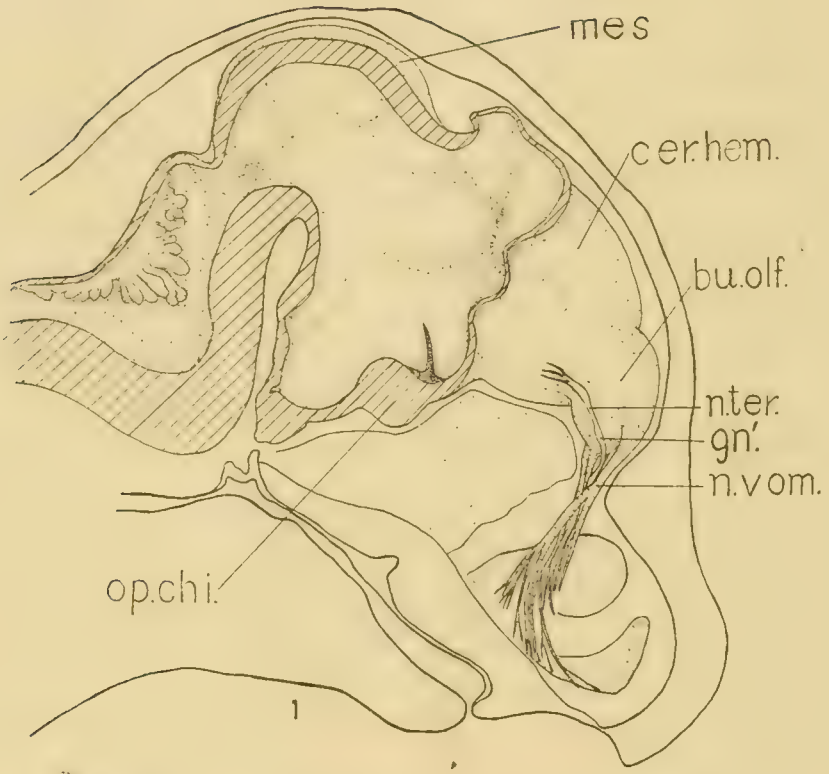
The embryos sectioned were cut in the sagittal plane or transversely, and were stained by various methods. The most generally satisfactory stain for older stages was found to be iron-hematoxylin, but some of the most instructive series were obtained by overstaining with Delafield's hematoxylin, followed by a counterstain of saturated aqueous orange G to which two drops of glacial acetic acid were added for each 50 cc. of stain.

The serial sections studied were as follows:

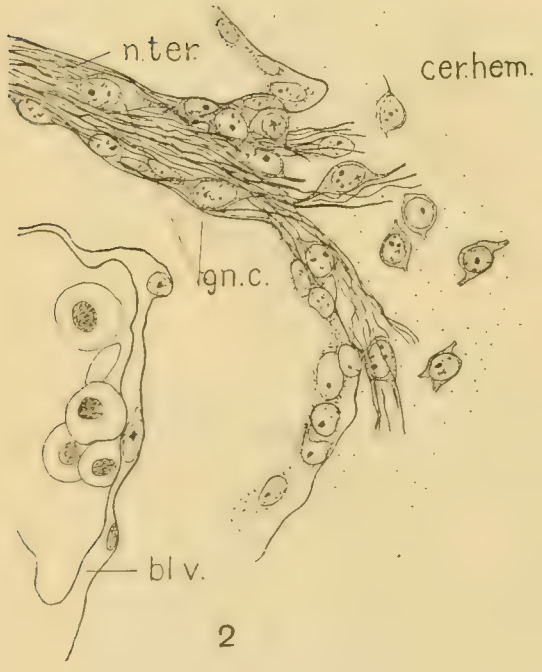
1 series 6-mm. embryo, sagittal, stained with iron-hematoxylin.
2 series 6.3-mm. embryo, transverse, stained with hematoxylin and Congo red.
3 series 7.5-mm. embryo, transverse, stained with hematoxylin and Congo red.
1 series 8-mm. embryo treated by the Cajal method, cut sagittally.
1 series 9-mm. embryo, sagittal, stained with hematoxylin and Congo red.
1 series 9-mm. embryo, sagittal, treated by Vom Rath method.
1 series 9.5-mm.-carapace-length embryo, stained with hematoxylin and Congo red, sagittal plane.
2 series 10-mm.-carapace-length embryos, sagittal, stained with iron-hematoxylin.
1 series 10.5-mm.-carapace-length embryo, sagittal, stained with hematoxylin and erythrosin.
1 series 11-mm.-carapace-length embryo, sagittal, stained with hematoxylin and Van Gieson's stain.
1 series 11-mm.-carapace embryo, sagittal, stained with hematoxylin and orange G.

DESCRIPTIVE

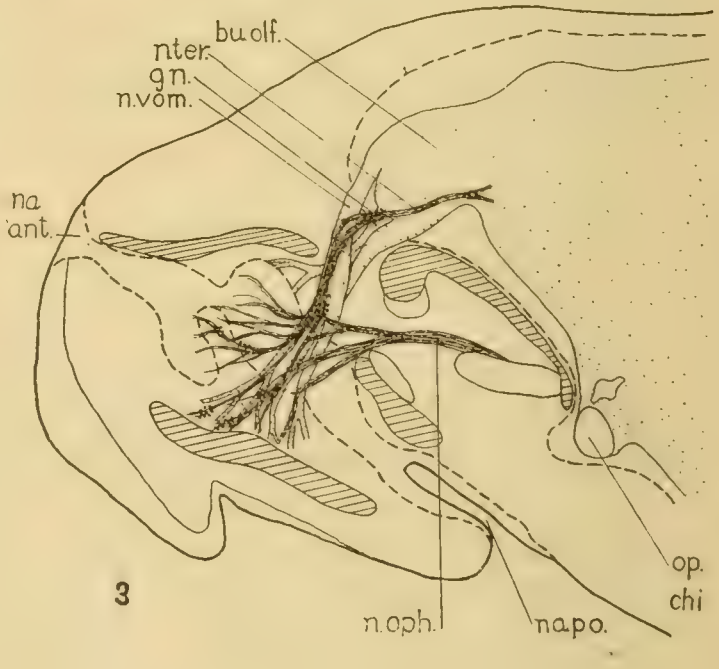
The nervus terminalis in the turtle has its origin by several small roots from the ventromesial surface of the forebrain, just caudad to the olfactory bulb. It can be demonstrated by dissection in suitably prepared material. Figure 1 represents a dissection of an embryo of 11-mm. carapace length, showing the left nervus terminalis and its relation to neighboring structures. In the specimen figured the rootlets were not evident until some of the overlying brain tissue had been removed by brushing. By this process three roots were demonstrated. In other dissections but two roots were brought to light, usually after some brushing.


mes
cer.hem.
bu.olf.
n.ter.
gn.
n.vom.
op.chi.

1


n.ter.
cer.hem.
gn.c.
bl v.

2


bu.olf.
n.ter.
gn.
n.vom.
na
ant.
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chi
n.oph.
na.po.

3

In sections of corresponding stages, cut in the sagittal plane, two or three roots were observed to enter the brain substance, but their fibers could be followed within the brain for only a short distance (fig. 2).

On following these roots distally from the brain, they are seen to unite (figs. 1 and 2) and a ganglionic swelling was invariably found just beyond their point of union. In the dissection figured it will be noted that the two more dorsal roots unite to form a single short trunk before entering the ganglion, while the ventral root enters the ganglion directly. In sections a number of ganglion cells (figs. 2 and 3) may be observed in this mass, but in none of the embryos examined did this ganglion appear to have as many cells as others located more rostrally, especially the one marked *gn.* (fig. 3).

Rostrally from the ganglionic mass which lies at the junction of the rootlets, the nerve continues as a compact trunk as far as the most anterior part of the olfactory bulb, midway between the

Fig. 1 Dissection of head of turtle embryo of 11-mm. carapace length to show the nervus terminalis and its relation to neighboring structures. The left lateral half of the brain is viewed from the mesial aspect. × ca. 15.

Fig. 2 Central roots of the nervus terminalis at their point of junction with the brain. Turtle embryo of 11-mm. carapace length. Hematoxylin and orange G stain. × 410.

Fig. 3 Reconstruction of the right nervus terminalis of turtle embryo of 11-mm. carapace length, stained with hematoxylin and orange G. The nervus terminalis is represented as lying on the medial surface of the vomeronasal nerve for all of that part of its course which is parallel to the latter. Part of its course, however, as shown in figure 1, is lateral to the vomeronasal nerve. × ca. 16.

ABBREVIATIONS

bl.v., blood-vessel
bu.olf., olfactory bulb
cer.hem., cerebral hemisphere
cr.cav., cranial cavity
gn., main ganglionic mass (ganglion terminale) of nervus terminalis
gn.', accessory ganglion terminale
gn.c., ganglion cells
gn.cl., ganglionic clusters
mes., mesencephalon
m.ob.do., dorsal oblique muscle
m.ob.ven., ventral oblique muscle
m.r.ant., anterior rectus muscle
n.olf., olfactory nerve bundles
n.oph., ophthalmic branch of V nerve
n.ter., nervus terminalis
n.vom., nervus vomeronasalis
na.ant., anterior naris
na.po., posterior naris
olf.epith., area of olfactory epithelium
op.chi., optic chiasma
ret., retina

dorsal (vomeronasal) bundle and the main bundle of olfactory fibers proper, as shown in figure 1. At this point it turns to follow these bundles, passing between them in such a manner that it could not be traced further by the method of dissection. Sections, however, reveal the further course of the main bundle of the nerve and indicate the presence of numerous ganglionic cells scattered along its trunk as it passes over the mesial surface of the olfactory bulb. These cells form clusters of various sizes. One of the largest of these clusters is no doubt indicated by the swelling (fig. 1, *gn.'*) shown in the dissection. Another and larger ganglionic mass is shown (fig. 3, *gn.*) at the point where the terminalis passes lateral to the vomeronasal bundle. This corresponds to the position usually occupied by the largest cluster of cells in the majority of the embryos which were sectioned.

From the position of this ganglion distally the nerve could not be followed further by the method of dissection, because its strands became too intimately mingled with those of the olfactory and vomeronasal nerves. Fortunately, however, in some of the series of the older stages studied, a differential stain was obtained by the method previously described, so that the terminalis bundles could be distinguished from those of the other two nerves and, further rostrad, from the fibers of the ophthalmic branch of the V nerve. This differentiation was aided by the fact that the olfactory and vomeronasal nerves appear as compact bundles of wavy fibers with few nuclei scattered among them. The strands of the nervus terminalis are smaller and much less compact and present relatively numerous sheath nuclei as well as larger ganglionic cells. Where the strands of the trigeminus were intermingled on the septum, they also had a characteristic appearance, apparently due to the process of myelination, as well as to differential staining. These characteristics, however, could not be noted with any degree of certainty in the smaller tracts, so that the reconstruction represented in figure 3 indicates only the larger bundles and their grosser ramifications.

In some of the preparations the nerve was seen to divide intracranially at the ganglion *gn.* into two strands, which, however, reunited to form a compact trunk before the nerve left the brain

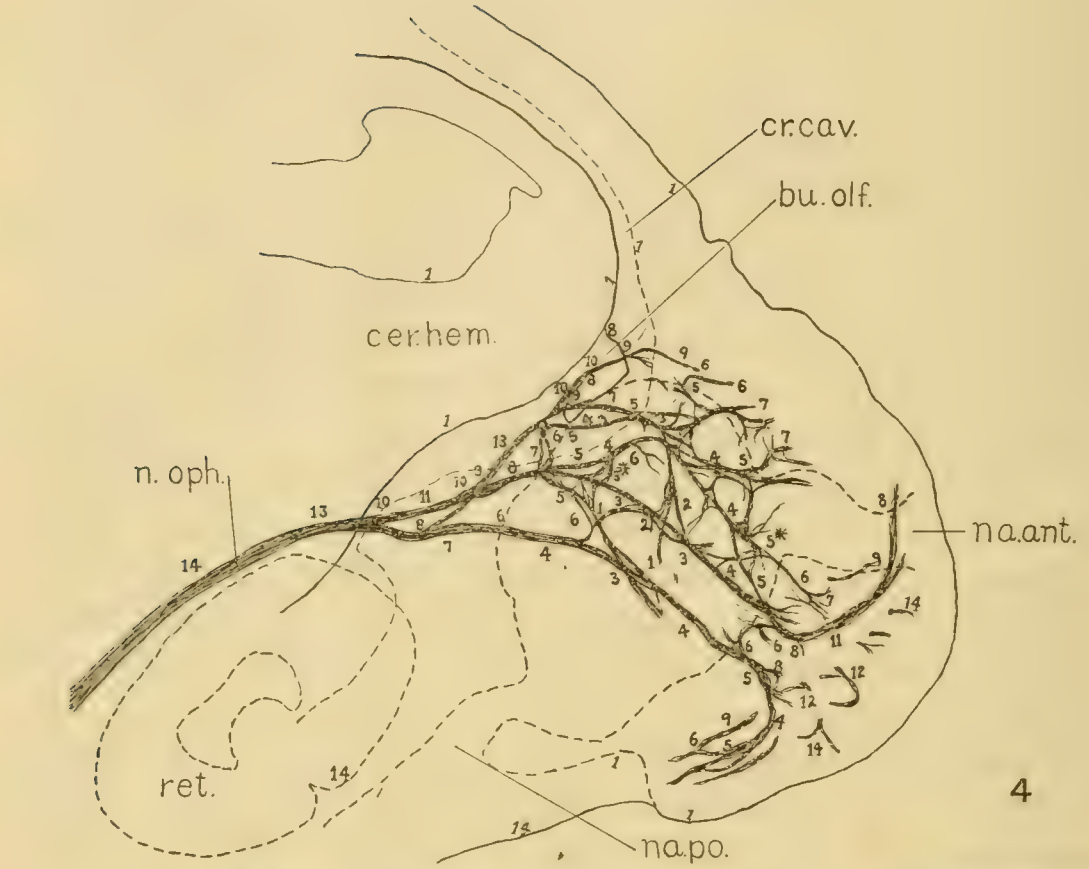
cavity. This condition is illustrated in figure 7. There were some indications of much finer strands also in this region, but they were not sharply enough differentiated from the olfactory strands to justify inclusion in the figure as part of an intracranial plexus of the terminalis.

After emerging from the cranial cavity, the nervus terminalis is composed of several well-marked strands which continue parallel with the vomeronasal bundles, mesial and in part dorsal, to the latter. A short distance from the point of emergence of the nerve, its strands begin to form a plexus over the nasal septum. Lack of silver preparations of the older stages made it impossible to follow this plexus for any considerable distance, especially in its more rostral part, where it becomes more complex due to entrance of fibers from the trigeminus. It was very evident that both the nervus terminalis and rami from the ophthalmic branch of the V nerve take part in the formation of a plexus on the nasal septum.

Clusters of ganglionic cells (fig. 3) were scattered throughout this plexus. Along the vomeronasal nerve there was a nearly continuous mass of cells from the point where the nerve turned ventrorostrally at the bulbus olfactorius, to the point where the more profuse spreading out of the septal plexus began. A somewhat similar arrangement of cells along the vomeronasal nerve was found by Johnston ('13) in Emys, but apparently the cells were not so numerous as in Chrysemys.

A fortunate Cajal preparation of an 8-mm.-total-length embryo gave a very clear demonstration that the trigeminus forms a more important portion of this septal plexus than might have been anticipated. As shown in figure 4, which represents a reconstruction from fourteen serial sections cut in the sagittal plane, the trigeminal portion of the plexus is formed by the ramifications of the ophthalmic nerve. The fibers were stained quite uniformly brown or black. They could be followed to the gasserian ganglion, with the beautifully stained cells of which they united.

The nervus terminalis in this preparation is represented by a few clusters of cells and some yellowish fibers. The cells could not with certainty be distinguished from the mesenchymal cells,



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but sections of embryos of approximately the same stage which were stained by other methods, indicate that the clusters are composed of ganglionic cells, which from their position no doubt belong to the nervus terminalis.

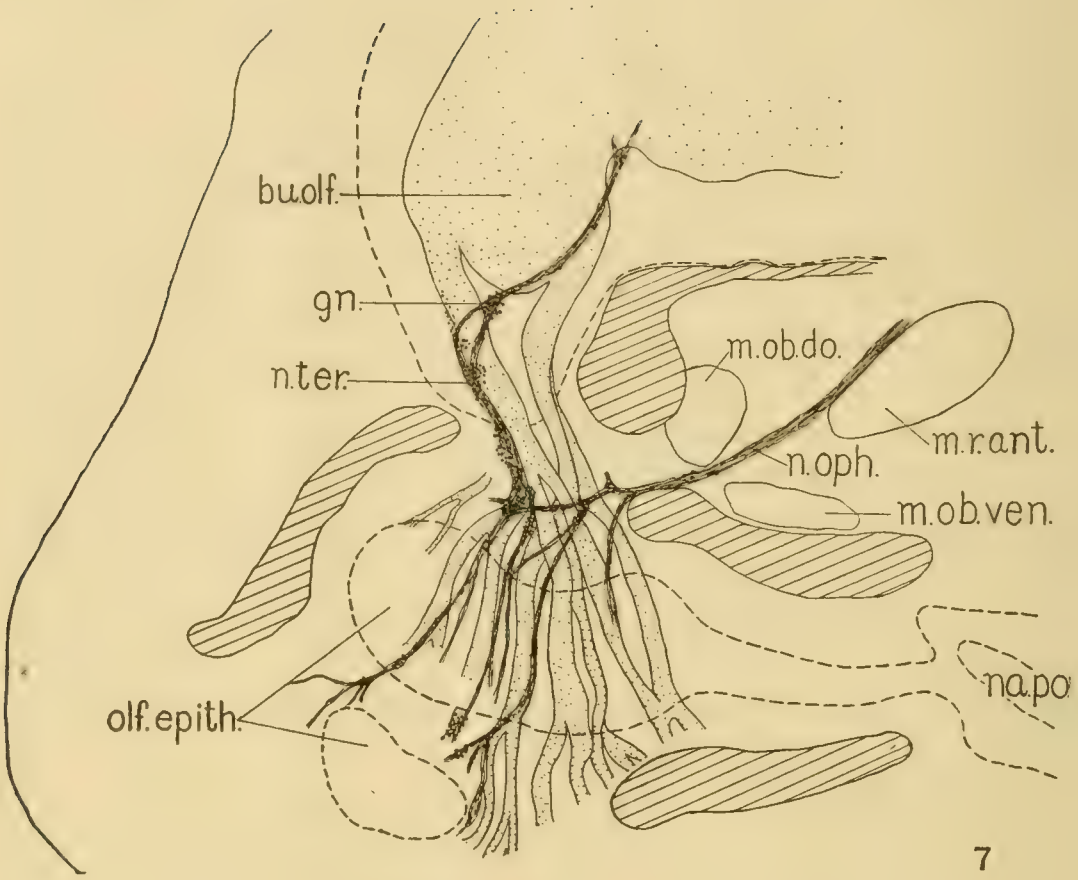
Some details of this plexus of the ophthalmic nerve are illustrated in figures 5 and 6. As shown in the figures, relatively small strands of fibers meet at nodal points, from which the individual fibers are redistributed to bundles diverging at various angles. No ganglionic cells could be observed about these nodal points at this early stage. In older stages, however, cell clusters of various sizes are numerous at the points where the ophthalmic nerve ramifies on the septum, and are found as far forward (figs. 3 and 7) as the most rostral part of the septum. It seems likely that the more rostral of these cells correspond to clusters of sympathetic cells described and figured by Willard ('15) in Anolis. The observation of Rubaschin ('03) of a 'ganglion olfactorii nervi trigemini' on one of the branches of the ophthalmic nerve in the chick appears also to be related.

At various points the branches of the trigeminus and of the terminalis become so intimately related that the two cannot be told apart, and the smaller strands of the plexus which continue from these points, appear to contain fibers from both nerves. As shown in figure 7, which represents a reconstruction from nine serial sections, from which the finer strands of the plexus are omitted, the nervus terminalis anastomoses with one of the larger branches of the ophthalmic nerve just dorsal to the vomeronasal nerve. At the point of anastomosis is a large ganglionic cluster. The two nerves had the characteristic different appearance, previously noted, proximal to their point of union, but their

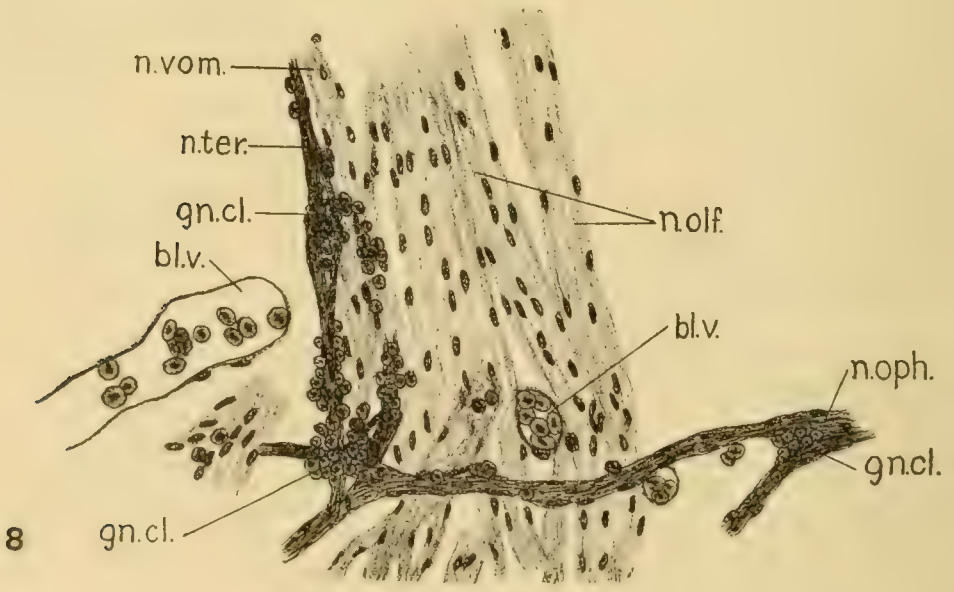
Fig. 4 Reconstruction of the septal plexus of the ophthalmic branch of the trigeminal nerve of a turtle embryo of 8 mm. greatest length, prepared by the Cajal method. The figure was reconstructed from sections 79 to 92 of the series. The numerals indicate the sections from which the adjacent structures were projected. For the sake of simplicity, section 79 is indicated by the numerals 1, section 92 by 14, and intervening sections accordingly. × 80.

Fig. 5 Portion of the plexus from section 81, at point marked 3* in the previous figure, to show details of structure. × 385.

Fig. 6 Portion of the plexus from section 88 of the series, at point marked 5* in the reconstruction, to show detail. × 385.


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gn.
n.ter.
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n.oph.
m.ob.ven.
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na.po

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n.vom.
n.ter.
gn.cl.
bl.v.
n.olf.
bl.v.
n.oph.
gn.cl.
gn.cl.

8

strands could not be told apart distal to this point. The relation of the two nerves and something of their appearance are represented more highly magnified in figure 8, but this does not show the slight although clearly apparent differentiation of staining which the preparations themselves reveal in the larger bundles. The trigeminal fibers appear coarser and more compactly collected into bundles than do those of the terminalis, but in the smaller strands these characteristics are not apparent, probably because of the small number of fibers composing them.

The olfactory and vomeronasal bundles, which are also represented in the figure, resemble each other in being composed of very delicate fibers with a characteristic wavy appearance which could not be well represented in the drawing.

Huber and Guild ('13) in the rabbit and Brookover ('17) in the human found indications of such an anastomosis of terminalis and trigeminal strands on the nasal septum, and the present writer ('18) partially demonstrated it in the cat. It is rather striking that three nerves, the terminalis, the olfactory (Read, '08), and the trigeminus, should each form a plexus on the nasal septum. Two of these plexuses overlap to a marked degree. The vomeronasal nerve is not plexiform, except as the large bundles composing it branch and reunite to a slight extent in their course to the vomeronasal organ. In this connection a statement from the previously cited paper of Ayers is of interest. He states: "The nasal chamber in man therefore contains the surface distribution of these three (terminalis, septalis, olfactorius) cranial nerves as well as the surface terminations of invading branches of a fourth and more recent cranial nerve, the trigeminus."

Fig. 7 Reconstruction from ten sections (175 to 185) of the series from which figure 3 was reconstructed, to show anastomosis between rami of the nervus terminalis and of the ophthalmic branch of the trigeminal nerve. × 44.

Fig. 8 Reconstruction from four of the sections (sections 182 to 185) included in figure 7, to show at higher magnification the anastomosis of one of the rami of the ophthalmic plexus with a bundle of the nervus terminalis. This figure also gives some idea of the appearance of the fiber bundles. × 180.

GANGLION CELLS

An effort to reach some conclusion as to the character of the ganglion cells of the terminalis in the turtle embryos was made by comparing them with cells of other ganglia, cranial, spinal and sympathetic. The gasserian, sphenopalatine, and ciliary ganglia were studied in the head region. The spinal ganglia, beginning with the first thoracic, and the sympathetic chain ganglia were studied in the body region. Measurements were made of the nuclei with the aid of an ocular micrometer, the results of which are indicated in table 1. The cells measured were taken at random. The only selection exercised was in measuring nuclei

TABLE 1

POSITION OF CELLS	NUMBER MEASURED	SIZE OF LARGEST NUCLEUS	SIZE OF SMALLEST NUCLEUS	AVERAGE SIZE OF ENTIRE NUMBER	REMARKS
		μ	μ	μ	
Periphery of spinal ganglia	48	12.3	8.2	9.8	Many unipolar.
Central part of same ganglia	52	8.2	5.3	7.3	Bipolar, approaching unipolar condition
Above combined	100	12.3	5.3	8.5	
Gasserian ganglion	52	11.4	7.0	9.0	Two sizes present, but small cells not many
Ciliary ganglion, large cells	50	12.4	7.0	9.0	Nuclei large in proportion to entire cell
Ciliary ganglion, small cells	22	7.9	5.3	6.4	
Above two combined	72	12.4	5.3	8.3	
Sympathetic chain ganglia	50	8.1	5.3	6.2	Five nuclei larger than 6.7 μ
Sphenopalatine ganglion	50	8.1	5.3	6.74	
N. terminalis, peripheral clusters	50	8.8	5.3	6.72	Some of these cells probably belonged to clusters related to the trigeminus
N. terminalis, central root clusters	30	8.4	5.3	6.7	Of the 80 nuclei measured in both peripheral and central clusters three were larger than 8.1 μ and three were smaller than 5.5 μ.

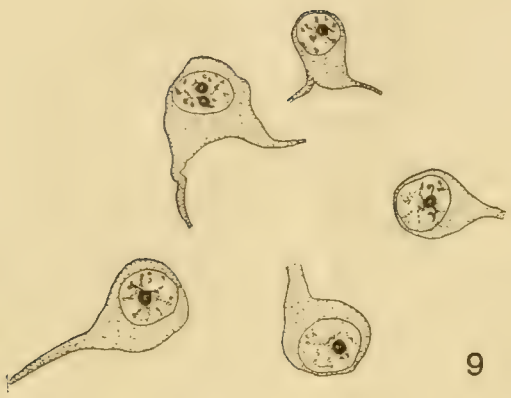
which were spherical or nearly so. In the case of some cells, especially many in the terminalis ganglia, the nuclei were so elongated that it was necessary to measure the greater and the lesser diameters and take the mean of the two.

All of the measurements and the drawings of the ganglion cells represented were made from a single embryo of 10-mm. carapace length, stained with iron-hematoxylin. This was done for the sake of uniformity, although the statements hold in general for all the embryos examined which were sufficiently advanced to show any pronounced differentiation of the various types of nerve cells. In drawing the figures the outlines of the cells and nuclei were traced with the aid of the camera lucida, and the same combination of lenses was employed in each case, so that the figures represent directly the variations in form and size of the various types.

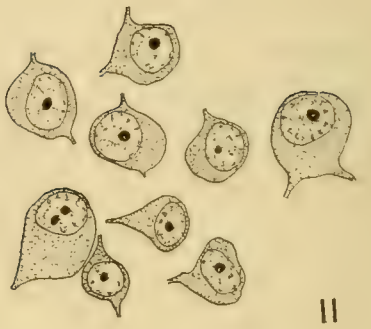
Comparison of embryos at different stages of development indicated that in embryos of 10-mm. total length, the spinal ganglion cells were on the average somewhat larger than those of the sympathetic chain ganglia. There was, however, but slight difference in the size of the individual cells within the spinal ganglia. The sympathetic chain ganglion cells had still much the appearance of indifferent cells. In the gasserian ganglion of embryos at this stage of development, many of the cells were larger than the spinal ganglion cells of the same embryo.

In embryos of 9.5 to 11-mm. carapace (15.5 to 17 mm. greatest length) there is a marked difference between the size of the largest sensory ganglion cells and those of the sympathetic chain ganglia. The latter (fig. 12) are of pretty uniform size, but the spinal ganglia and, in less marked degree, the cranial ganglia, showed two fairly distinct sizes of cells. The larger type in the spinal ganglia (fig. 9) was found near the periphery of the ganglion. Many had already reached the unipolar condition, but others showed various transitional stages from the primitive bipolar cells.

Of one hundred cells measured from three different ganglia, one in the thoracic region, one in the lumbar, and one in the sacral, the largest nucleus had a diameter of 12.3 μ and the



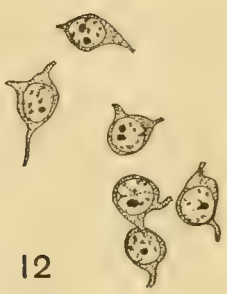
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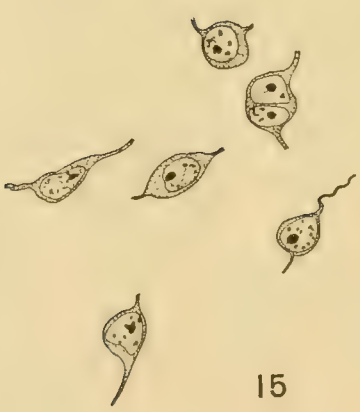


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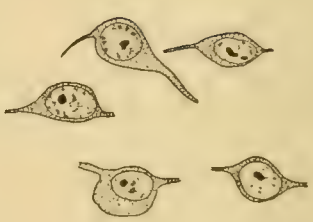
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smallest was 5.3 μ in diameter. The average size of the entire one hundred nuclei was 8.5 μ. These cells were divided into two groups, those with a nuclear diameter greater than 8.2 μ and those whose nuclear diameter was less than this, down to 5.3 μ which was the smallest found. While this division was somewhat arbitrary, there was sufficient ground for it in the character of the cells, aside from their size, to make it appear justifiable. The smallest cells (fig. 10) had considerably less cytoplasm surrounding the nucleus, both relatively and actually. Only various stages of the bipolar condition were observed in these smaller cells. They were found closely packed together in the central portion of the ganglion, while the larger cells were nearer the periphery.

As indicated in the table, the fifty-two cells which showed a nuclear diameter less than 8.2 μ had an average diameter of 7.3 μ. The forty-eight cells whose nuclear diameter was greater than 8.2 μ were found to have an average nuclear diameter of 9.8 μ.

The smaller cells (fig. 10) may correspond to the small ganglion cells found in the spinal ganglia of mammals by Dogiel ('08), Ranson ('12), and other workers, but it seems likely that some of them at least represent cells of the larger type which have not

Fig. 9 Cells from the peripheral portion of the first thoracic spinal ganglion of a turtle embryo of 10-mm. carapace length. Iron-hematoxylin stain. × 500.

Fig. 10 Cells from the central portion of the same ganglion from which figure 9 was drawn. Turtle embryo 10-mm. carapace length, iron-hematoxylin stain. × 500.

Fig. 11 Cells from the gasserian ganglion of turtle embryo of 10-mm. carapace length. Iron-hematoxylin. × 500.

Fig. 12 Cells from the third thoracic sympathetic chain ganglion of turtle embryo of 10-mm. carapace length. Iron-hematoxylin. × 500.

Fig. 13 Cells from the ciliary ganglion of turtle embryo of 10-mm. carapace length. Cells of smaller type indicated at *A*, and the larger type at *B*. Iron-hematoxylin. × 500.

Fig. 14 Cells from the sphenopalatine ganglion of turtle embryo of 10-mm. carapace length. Iron-hematoxylin. × 500.

Fig. 15 Cells from the peripheral cell clusters of the nervus terminalis of a turtle embryo of 10-mm. carapace length. Iron-hematoxylin. × 500.

Fig. 16 Cells from the central root clusters of the nervus terminalis of a turtle embryo of 10-mm. carapace length. Iron-hematoxylin. × 500.

yet reached the same degree of development. This view appears to be favored by the extremely crowded condition of these cells within the ganglia. This would appear to result in a reduction both of the amount of space and of nourishment which the individual cell may obtain, thus retarding its growth. The fact that the larger cells were found near the periphery of the ganglia, where there would appear to be space for greater expansion of the cells during growth as well as more abundant nourishment, may account for their larger size at this stage of development. There were also cells of intermediate size between the two groups, but these were not so numerous as the cells of either group.

In the gasserian ganglion (fig. 11) of the older stages the number of small cells was not so great as in the spinal ganglia, but here also some were present, as the figure indicates.

The sympathetic chain ganglia of the older stages, as in the younger, contained cells of rather uniform size and appearance (fig. 12). Two or three processes were observed on most of the cells which were examined on this point. The nuclei were smaller than those of the small spinal ganglion cells, showing an average size for fifty cells of 6.2 μ, as compared with 7.3 μ for the latter type. The relative amount of cytoplasm surrounding the nucleus appeared to be about the same in the two types. No difference in the size of the peripherally located cells, as compared with those situated nearer the centers of the sympathetic ganglia, was observed.

The ciliary ganglia showed two groups of strongly contrasting cells. Without entering into a detailed description of this ganglion or attempting to review the large amount of literature which has accumulated concerning it, the present purpose will be served by calling attention to Carpenter's ('06) excellent study of it in the chick and adult fowl. He finds two distinct sizes of cells. The smaller cells were arranged in a definite group on the dorsal side of the ganglion. This group composed about one-third of the entire mass.

A similar group of small cells (fig. 13, *A*) was present in many of the turtle embryos, but not in all, studied by the present

writer. In all cases, however, whether or not arranged in a distinct group, small ganglionic cells were found in the ganglion. These differed not only in size, but also in form, from the larger more typical cells. Carpenter considers the large cells in the chick to be derived from the midbrain and to have migrated along the oculomotor nerve to the ciliary ganglion. The small cells he believes to be sympathetic cells which have migrated forward along the ophthalmic nerve.

Fifty of the large cells were measured. The largest had a nuclear diameter of 12.4 μ, the smallest of 7 μ. The average nuclear diameter of the fifty cells was 9 μ. While the size of these nuclei approached that of the large spinal ganglion cell nuclei, and the largest found in the ciliary ganglion even exceeded the largest observed in the spinal ganglia, the amount of cytoplasm surrounding the nuclei of the ciliary ganglion cells was considerably less, as may be seen by comparing figures 9 and 13, *B*. The actual size, therefore, of the ciliary ganglion cells was somewhat less than that of the large spinal ganglion cells.

Twenty-two cells of the smaller type were measured. These were found in several adjacent sections, forming a distinct mass in the embryo on which the measurements were made. The largest of these cells had a nuclear diameter of 7.9 μ. The smallest was 5.3 μ, and the average of the twenty-two was 6.4 μ. As figure 13 (*A*) indicates, there were also differences in the form of the cells and in the amount of cytoplasm surrounding the nucleus, as compared with the large cells.

There remain the cells of the sphenopalatine ganglion, which form a relatively small cluster. These cells are of quite uniform size and structure (fig. 14) with spheroidal, eccentrically located nuclei. Most of them were in the primitive bipolar stage of differentiation. Except that there was less individual variation among these cells than among those found in the clusters of the nervus terminalis, to be described, the sphenopalatine cells may be said to resemble the terminalis cells more closely than any of the other ganglionic cells studied. Of fifty sphenopalatine cells which were measured, the largest had a nuclear diameter of 8.1 μ. The smallest was 5.3 μ, and the average for the fifty nuclei was 6.74 μ.

With these measurements as criteria, a comparison may be attempted between the ganglionic cells of the nervus terminalis and these other cells of well-recognized function, with the purpose in mind of throwing more light on the relationship of the nervus terminalis.

Many of the terminalis cells (figs. 15 and 16) have a peculiar elongated form not met with elsewhere in the ganglia which were subjected to observation. This is well illustrated in some of the cells represented in figure 15. Only two processes were observed in any of these cells, and these were in most cases continuous with the longer axis of the cell. In view of the findings of McKibben ('14) in the dogfish and of the various authors who have made observations on the ganglion cells of the terminalis in the mammals, it seems likely that this bipolar condition is developmental in the turtle.

The nuclei were elongated in many cases and many were also considerably distorted otherwise, as the figures indicate. This was more frequently the case in the peripheral clusters than in those within the cranial cavity. These conditions made the terminalis cells more difficult to measure, so that the results obtained represent the mean of several measurements on many of the nuclei. In order to determine the difference in size, if any, between the cells which were located in the ganglionic swelling near the central roots of the nerve and those along the olfactory and vomeronasal nerves outside the cranial cavity, the measurements were tabulated separately for the two groups. Thirty cells from the centrally located ganglion (fig. 16) indicated an average nuclear diameter of 6.72 μ, the largest being 8.4 μ, and the smallest 5.3 μ (table 1). The fifty cells of the peripheral clusters (fig. 15) which were measured indicated an average nuclear diameter for the entire number of 6.7 μ, practically the same as that of the centrally located cells. The largest nucleus of the peripheral clusters was 8.8 μ in diameter, the smallest was 5.3 μ. A larger number of measurements was not made because the variation in size was not pronounced. Only three of the eighty nuclei measured were larger than 8.1 μ, i.e., more than 1.4 μ larger than the average. This amount indicates the

variation above the average size which the smallest cells, which had nuclear diameters of 5.3 μ, showed below the average size. Three cells of this smallest size were found to be included among the eighty measured.

Reference to the table will indicate that the cells of the gasserian, spinal, and ciliary ganglia have an average nuclear diameter of 8.3 μ or more, even when the smaller cells, which in the spinal and ciliary ganglia may with some show of reason be considered as a separate group, are included with the larger in computing the average. If the two types are considered separately, the average size of the larger cells, which may be considered as the typical cells in the three ganglia under consideration, is considerably increased, being 9 μ for the ciliary ganglion and 9.8 μ for the spinal and gasserian ganglia. The sympathetic chain ganglia, the small cells of the ciliary, and the sphenopalatine ganglion cells, together with those of the nervus terminalis, have an average nuclear diameter of approximately 6.7 μ or less.

When these facts are considered in connection with the difference in form of the cells of the terminalis clusters, and their manner of distribution in more or less irregular clusters instead of in the compact ganglia of the sensory nerves, it seems reasonable to conclude that the majority of cells at least, which are found in the ganglionic clusters of the nervus terminalis in the turtle, are related to the sympathetic nervous system.

The writer's previous work on various mammals, together with Huber and Guild's ('13) comparison of terminalis cells with those of the gasserian ganglion in the rabbit, points in the same direction for mammals, as does the work of McKibben ('14) on the terminalis ganglion of Mustelus, for the selachians, and certain of Brookover's observations in ganoids for that group of vertebrates.

At this point attention may also be called to the position of some of the ganglion cells at the point of entrance of the nerve roots into the brain, as illustrated in figure 2. The position of these cells is interesting. As shown in the figure, some of them appear to be migrating outward from the brain wall. Both cells and nuclei are elongated, and the cells lie just at the border of

the limiting membrane of the brain. The limiting membrane in this region is bulged forward to a considerable extent. Some of the cells appear to have passed beyond this membrane, at least they lie outside of it, but their processes extend into the brain substance. Similar cells were found along the entire course of the nerve between the entrance into the brain substance of the central roots and the rostral end of the olfactory bulb. The position of a few of these cells is indicated in figure 7.

These cells strongly suggest migratory cells, such as have been pointed out in the ventral roots of the spinal nerves of the turtle embryo by Kuntz ('11 b) and of the pig embryo by Carpenter and Main ('07). These writers conclude that the cells which migrate from the cord into the ventral nerve roots have a part in the formation of the sympathetic ganglia, together with migratory cells from the dorsal root ganglia. Gaskell ('16) takes a similar view.

A condition similar to that illustrated in figure 2 was observed in a kitten one day old, and was illustrated in the previous paper (fig. 23) on the nervus terminalis in mammals. No comment was offered at the time on the point now under consideration, since the observation had not been repeated in more than one preparation. Such a relationship of cells is present in all of the turtle embryos which are far enough advanced in development to show distinct differentiation of ganglionic cells.

CONCLUSION

The nervus terminalis of the turtle takes part in the formation of a plexus on the nasal septum, comparable to that found in mammals. In the turtle fibers from the trigeminal nerve form a more important share of this plexus than has yet been demonstrated to be the case in mammals.

There is a pronounced resemblance of the cells of the ganglionic clusters of the nervus terminalis to the cells of the various sympathetic ganglia. This resemblance is apparent not only in size, but also in their structure and manner of distribution and to some extent, so far as the developmental evidence goes, in their apparent origin as migratory cells from the central nervous system.

The ganglionic cells in the regions which appear to be occupied solely by fibers of the terminalis, i.e., without intermingling of trigeminal fibers, resemble migratory cells which are found along the ophthalmic branch of the trigeminal nerve so closely that they cannot be told apart. It is probable that the ganglionic clusters of the septal plexus are composed of cells from both sources, as the plexus itself is composed of nerve fibers from the two sources.

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Resumen por el autor, C. G. MacArthur.
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Cambios químicos cuantitativos del cerebro humano durante el crecimiento.

Durante el crecimiento, las proteínas, fosfátidos, sulfátidos, cerebrósidos, colesterol y sólidos totales aumentan en tanto por ciento. Solamente hay un ligero cambio en el tanto por ciento de los extractivos, mientras que el agua decrece regularmente hasta la edad adulta. La mayor parte de los compuestos cerebrales, con la excepción de los cerebrósidos y sulfátidos, se descomponen con mayor rapidez en el recién nacido, y cuando se suman diariamente, su cantidad en miligramos es la siguiente: agua 3270, sólidos 494, lipinas 165, fosfátidos 85, colesterol 70, sulfátidos 7.7, cerebrósidos 1.9, proteinas 186, extractivos orgánicos 100, extractivos inorgánicos 44, azufre 2.3, fósforo 8.5. Probablemente durante el crecimiento la médula espinal contiene la mayor cantidad, en tanto por ciento, de sólidos totales, lipinas totales y de cada lipina, pero la menor cantidad de proteína, extractivos y agua. El cerebro difiere poco de la médula, mientras que el cerebelo difiere mucho. El análisis químico está de acuerdo con la existencia de tres estados en el crecimiento del cerebro: 1) Aumento del número de células; 2) su crecimiento, incluso el de los cilindro ejes; 3) la medulación. Aunque la cantidad absoluta de cada uno de los componentes sumados es mayor durante el periodo medio de crecimiento (en el recién nacido), tiene lugar un aumento mayor de crecimiento en el tejido más jóven. El crecimiento del cerebro no es necesariamente autocatalítico. El cerebro entero, del mismo modo que cada componente, aumenta con el desarrollo, tal como sucedería a una masa determinada de protoplasma que construye más material semejante a él en un ambiente que cada vez es más desfavorable. Parece una necesidad lógica que ni aún depende de la vida.

Translation by José F. Nonidez
Carnegie Institution of Washington

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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QUANTITATIVE CHEMICAL CHANGES IN THE HUMAN BRAIN DURING GROWTH

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THREE CHARTS

It seemed desirable to develop a more complete growth series of quantitative determinations of the important constituents in the human brain than has heretofore been published (Koch-Mann, '07–'08). During fetal life and infancy these changes are most interesting, but least studied. The chemical differentiation during growth in young pigs (Koch, '13) and young rats (W. and M. L. Koch, '13) has received attention, but early human life has been curiously neglected.

METHOD OF ANALYSIS

The method of analysis is essentially the same as that used by others in quantitative brain work.[1] The outline on page 446 gives the main points.

LIMITATIONS AND ERRORS

It needs to be kept in mind that disease caused the death of the people whose brains were analyzed. Though the brain in no case showed appreciable lesions, yet it is always possible that chemical alterations might have taken place before death.

Only one brain (except in the case of the three-month fetal brains) was analyzed for each of the ages given. Of course there is no guarantee that each was an average brain. Moreover, we have, because of the few analyses, no means of finding out the

[1] For detail of method and formulae for calculation of results see Koch, W., J. Am. Chem. Soc., 31, 1329, ff. 1909; Koch, M. L., and Voegtlin, C., Hyg. Lab. Bull. No. 103, p. 67. 1916.

Moist brain tissue: Add alcohol and extract alternately with alcohol and ether

EXTRACT[2] (FRACTIONS 1 AND 2)		RESIDUE (FRACTIONS 3 AND 4)	
Evaporate to dryness, emulsify with water, precipitate with $CHCl_3$ in 0.5 per cent HCl solution		Dry, weigh, and extract with hot water.	
Precipitate (fract. 1): (Colloidal)	Filtrate (fract 2): (Crystalloidal)	Filtrate (fract 3): (Crystalloidal)	Residue (fract. 4): (Colloidal)
Lipoids *Phosphatids* *Cerebrosides* *Sulphatids* (*Cholesterol, etc.*)	*Organic constituents:* Hypoxanthin Tyrosin Leucin Urea Inosit Taurin Peptones Sarcolactic acid *Inorganic constituents* (see fract. 3)	*Inorganic constituents:* Ammonium Iron Sodium Potassium Calcium Magnesium Chlorides *Organic constituents* (see fract. 2)	*Proteins* Nucleoprotein (a) Nucleoprotein (b) Neurokeratin
Lipoid sulphur	*Neutral sulphur*	Inorganic sulphur (*Sulfates*)	*Protein sulphur*
Lipoid phosphorus	*Organic extractives* *Phosphorus*	Inorganic phosphorus (*Phosphates*)	*Protein phosphorus*
Lipoid nitrogen	Organic nitrogen	Inorganic nitrogen	Protein nitrogen

[2] Substances *in italics* were determined in this investigation.

average deviation. Not until many such series have been developed shall we be able to state what normal brain growth really is. To a certain extent, a smooth curve averages the data, but this may introduce larger errors than it attempts to average. There is no way of knowing definitely that a curve should be regular as given or made up of a series of waves of different sizes. This also can be determined only by a larger number of investigations.

If the thirty-five-year-old brain is normal, there is a rather wide range of variability. It will be noticed that the total solids in this brain are 5 per cent higher than in the other adult

brains. This cannot be an analytical error, because the same amount was obtained in two analyses made at different times in the series.

The brain marked '8–24 mo.' was labeled '2 years' when sent for analysis. There seems to be no good reason for questioning the accuracy of this information. However, the weight of the brain indicates an infant of a few months. Its water content suggests an infant of about eight months, while some of the phosphatids would favor a slightly greater age, as would the weight of cerebellum and stem. Very likely this brain was two years old, but subnormal. In spite of the uncertainty concerning the brain, it is included in this series because it was the only one of this age available, but it is considered with the greatest reservation in forming general conclusions.

No brains about five and twelve years of age could be obtained. This leaves the series incomplete.

Unfortunately, it was not known until the investigation was nearly finished that the method used for sulphur determination gave low results. This vitiates to a certain extent the reports on the various forms of sulphur, the sulphatids, and because of the methods of calculating the data, the cerebrosides, and the undetermined cholesterol. It was planned to make direct cholesterol estimations, but the series took so much longer than expected that these estimations were omitted.

Analyses 7 and 10 were made together. A combination of circumstances rendered their phosphatid determinations somewhat unreliable. If the solutions to be precipitated by chloroform and hydrochloric acid become too warm and are allowed to stand too long, the phosphatids are incompletely precipitated. This gives not only an error in phosphatids, but, by difference, in 'cholesterol, etc.' and in extractives.

Many ways were found of improving the method after beginning this series, but they could not be adopted because of the effect on comparisons of results. One always sees many ways of improving an investigation after it is finished, and that is unusually true of this investigation.

DESCRIPTION OF MATERIAL

Dr. H. Gideon Wells, of the Pathological Department of the University of Chicago, very kindly arranged to help us in this investigation. Without his cooperation, this series would have been very incomplete.

Upon receipt of a brain the meninges and blood were removed from the brain and it was divided into forebrain, cerebellum, and brain stem, and each division weighed. Samples were then taken and placed in enough 95 per cent alcohol to make the concentration 85 per cent alcohol. The specimens were as follows:

Three-month fetus. Male. Two three-month fetuses, referred to as normal, were united in order to furnish material enough for one good analysis. These brains were not divided into forebrain, cerebellum and brain stem.

Seven-month fetus. Female. The mother of this stillborn fetus entered the hospital five days before the delivery with signs and symptoms of placenta praevia. A brain of this age also gives too small amounts if separated into divisions, so such separation was not made.

One-month child. Male. This child died of bronchopneumonia. The forebrain was separated from the rest of the brain, the cerebellum and brain stem were analyzed together because there was not enough in either to make a satisfactory separate analysis.

Three-month child. Male. Died of bronchopneumonia and marasmus.

Eight to twenty-four month child. Male. Though there was no record of this child having been abnormal, the brain was found to be decidedly underweight for the two-year age reported. The child may not have been two years old, but younger. More likely it was subnormal.

Twenty-one year adult. Male. Died of pneumonia. The autopsy did not take place for three days after death, but the weather was cool, so the brain was in good state of preservation when received.

Thirty-three year adult. (negro). Male. Died of acute pneumonia. No other disease was present.

Thirty-five year adult (Hungarian). Male. Cause of death not reported. Brain was very high in solids, but not pathological in any evident way.

Sixty-seven year adult. Male. Died of tuberculosis.

The weights of the different divisions obtained from these brains were as follows:

Whole brain: Weights of divisions in grams

	FETUS 3 MONTHS	FETUS 7 MONTHS	CHILD 1 MONTH	CHILD 3 MONTHS	CHILD[3] 8 MONTHS	ADULT 21 YEARS	ADULT 33 YEARS	ADULT 35 YEARS	ADULT 65 YEARS
Whole brain.....	17.08	119.0	457.4	585.2	492.5	1122.4	1221.3	1158.3	1297.9
Forebrain.....			395.0	514.0	409.0	950.0	1026.0	986.0	1075.0
Left.....			200.0	263.0	206.0	485.0	516.0	510.0	535.0
Right.....			195.0	251.0	203.0	465.0	510.0	476.0	540.0
Cerebellum.....			(37.4)[4]	42.6	48.2	111.4	130.6	110.8	145.4
Brain stem.....			(25.0)[4]	28.5	35.3	61.0	64.7	61.5	77.5

[3] See section on Limitations for explanation.

[4] Analyzed together because of small amounts of each.

DISCUSSION OF RESULTS

Water and total solids

The water determinations show that though the absolute amount increases (table 12, fig. 1), the percentage of this constituent decreases continually (table 10) until growth is completed. The relative amount of water present is an indication of the rate of activity. Water, like the inorganic salts and the simpler organic substances (table 10), decreases relatively rather regularly with the approach to adult condition and its decrease in rate of metabolism.

The percentage of total solids of course varies inversely with that of water. The increase is largely due to the formation of the colloidal substances. They increase in absolute amounts (table 12, fig. 1) and in percentage (table 10), while the simpler molecules, as a rule, decrease in percentage, but increase in absolute quantity. It will be noticed from table 13 and figure 2 that the solids are formed most rapidly soon after birth; at least 0.5 grams a day are then being added. This coincides with the period of most rapid myelination.

It does not necessarily follow that this is the time of greatest protoplasmic activity, because under other conditions the products of the reaction may be removed, while during the period of myelination a part may form the sheath.

It needs to be remembered that the substances produced in myelination are not to be thought of as active protoplasmic

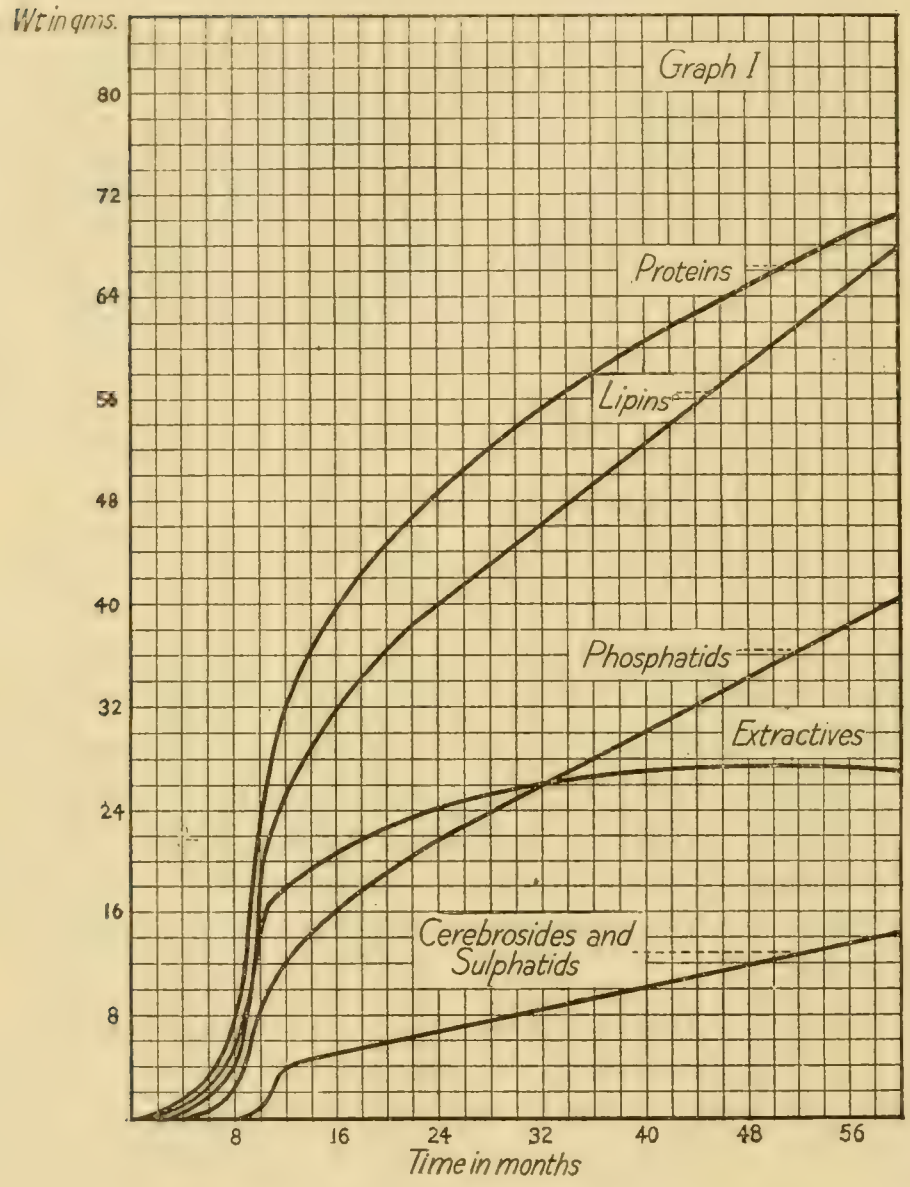


Fig. 1

compounds in the same way as the extractives, certain lipins, and nucleoproteins. This is true whether one considers the sheath as nutritional or insulating in function. In comparing nerve activity with other tissues, it would probably be more exact, therefore, to use data on the axis cylinders and not the whole nerve (Donaldson, '16).

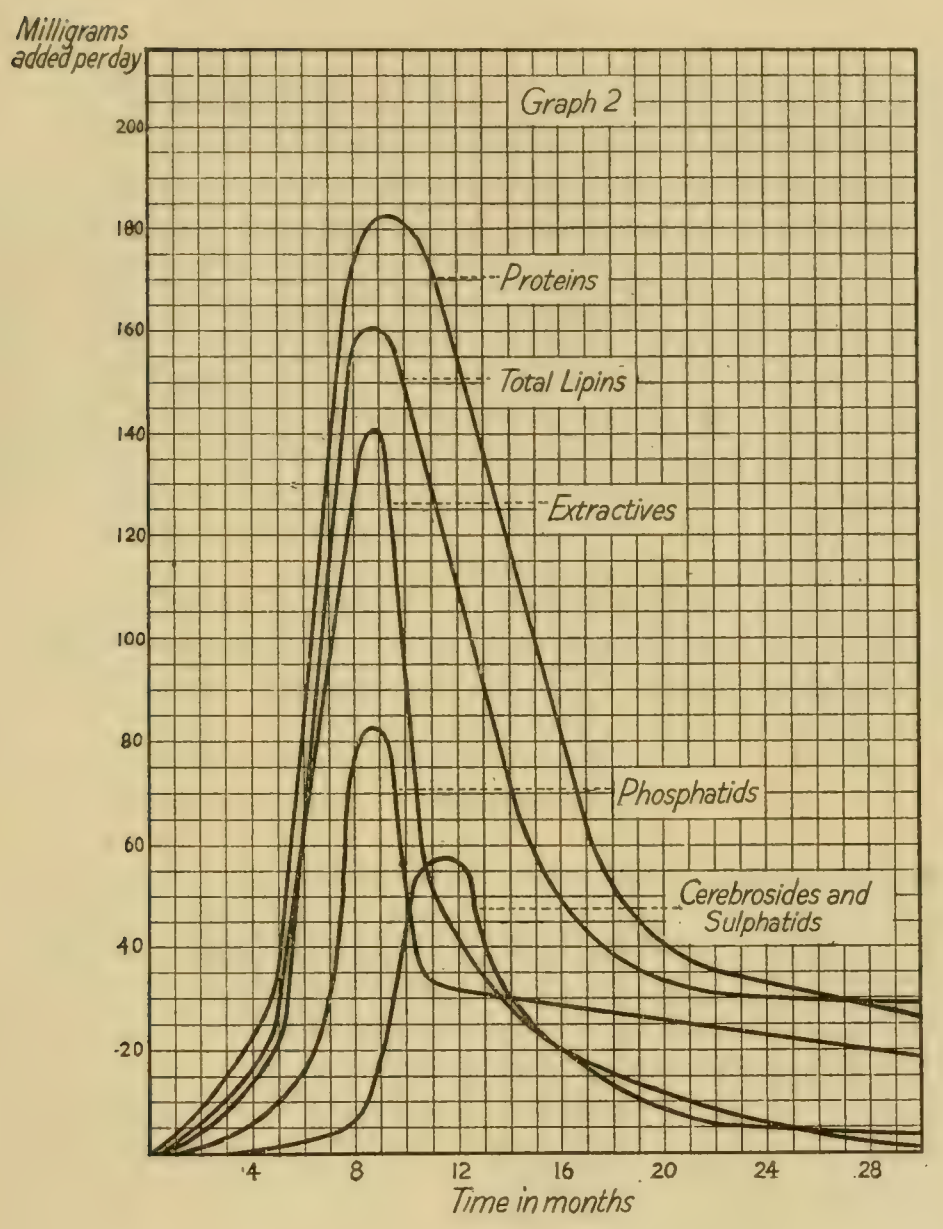


Fig. 2

Phosphatids

Lecithin (MacArthur and Darrah, '16), cephalin (MacArthur, '16), sphingomyelin and myelin are the principal phosphatids found preformed in the brain. Some or all of these compounds are present from very early fetal life (table 1). They gradually increase (table 12) until myelination becomes rapid, then they are formed at the maximum rate of 0.1 gram per day (table 13). They continue to be formed rapidly until two years of age; after

this the rate decreases to adult age. During adult life they probably increase very slowly and are one of the colloidal factors to be considered in retarding metabolism in old age.

We have some reasons for believing that lecithin is the phosphatid largely found in the nerve cells (Cowdry, '14). It looks as though it were rather closely associated with the nucleoproteins in carrying on vital activities. Cephalin is probably present in both the cells and axis cylinders, though more largely in the latter. Very little is known about sphingomyelin, but it it is probably largely to be found in the sheaths. It would be very interesting to study the increase in each of these phosphatids during growth.

Lecithin, and especially cephalin, because of their auto-oxidation characteristics, are believed to be closely related to nerve-tissue oxidation (Signorelli, '10; MacArthur and Jones, '17).

Cerebrosides

Phrenosin (Levine and Jacobs, '12) and kerasin (Rosenheim, '13), the two brain cerebrosides, may be parts of an unstable complex made up of sulphatid, phosphatid, and cerebroside. If this is true, the data in this paper would indicate that with growth this complex increases in complexity (fig. 1), because the cerebrosides as analyzed do not appear until birth (tables 2, 5, and 8), when myelination becomes the dominant brain activity. This may mean that they are in some way dependent on the presence of other constituents for their production. They are peculiar in the rapidity with which they assume such a prominent place in developing nerve tissue.

The cerebrosides are probably more directly related to sheath formation than any other constituent (Smith and Mair, '12–'13). Their maximum rate of formation does not occur as early as in the case of the other brain constituents (at four months instead of at birth) (table 11). Then about 0.025 gram are added each day.

Sulphatids

Because of uncertainty concerning the sulphatids found in the brain, the report for this constituent is open to question. It is assumed, however, that it is a cerebroside and phosphatid fastened together by a sulphate radicle (Koch, '10). The sulphatids are very closely related to the cerebrosides in physiological function and anatomical distribution. The sulphatids seem to be more fundamentally necessary because they are found earlier (table 10). They may be related to conductivity in axis cylinders. Small amounts are present in very early fetal life (tables 1, 4, and 6). Soon after birth the amounts formed are greatest (table 12), but there is no time during life when this compound is not being produced. Probably it is concerned in the rivalry between structure and function, helping the former to victory in stability of activity, and finally in death. This is one of those substances so necessary for highly specialized brain work, but so detrimental to continued growth.

Proteins

The most important protein of the brain, because of its greater lability, is probably nucleoprotein *a* (McGregor, '17). One would expect it to be associated with the vital functions. It probably is a combination of the globulin *a*, globulin *b*, and the nucleoprotein of an earlier worker (Halliburton, '94). Nucleoprotein *b* is much more stable and may be the protein of the chromatin and Nissl bodies, thus related to the hereditary quality of the nerve cell. Neurokeratin is stable and probably is connected with the structure of the nerve sheaths. It is highly important to know how these different proteins increase with growth, but we have only indirect evidence of what these changes are. From the data on total protein, protein phosphorus and protein sulphur (table 11), we can get an idea of what is happening, however, Thus indirectly we can suggest that neurokeratin approaches a maximum percentage at two years of age, but is present in very small amounts in even early fetal life. Nucleoprotein *b* is probably present in largest percentage amounts in

early fetal life, but continues to increase in absolute amounts (table 12) until maturity, when there is about twice as much as of nucleoprotein *a* and half as much as of neurokeratin (McGregor, '17). Nucleoprotein *a* possibly is always present, but probably is largest in percentage amounts when nerve growth and activity are greatest. It would not do even to guess how these last two proteins are distributed in the brain.

The total protein curve (fig. 1) indicates that some particular protein (possibly nucleoprotein *b*) is an especially important factor in the subsequent growth of the brain. It seems to lead in the increases that take place.

Extractives

The separation of extractives into organic and inorganic, as given in the data, is of but little value because of the fact that such a separation, based on the solubility of organic constituents in alcohol and the insolubility of the inorganic ones in alcohol (or on the residue after ignition), is very unreliable. The data given are merely suggestive. However, the determination of total extractives is rather accurate. Inosit, urea, leucin, tyrosin, taurin, hypoxanthin, and peptones are a few of the organic substances present in this fraction. In general it may be stated that the larger the percentage of these simpler crystalloidal molecules, the more rapid the metabolism and the younger the tissue. Various inorganic salts of sodium, potassium, ammonia, calcium, magnesia, and iron have about the same significance. While the rate of growth is high, these constituents are present in larger percentage amounts (table 11), but with a decrease in rate of development they rapidly decrease in rate of formation, until after two years of age they are but very slowly increased in absolute amount (table 12).

They are present in larger amounts in cells than in axis cylinders. Potassium salts and chlorides are supposed to be related to nerve conductivity (Alcock and Lynch, '11).

In drawing conclusions concerning the rate of activity of nerve tissue from the percentage amounts of extractives, one needs to

remember that it may be more accurate to leave the sheath substances out of the reckoning. The calculations would then be based on the assumption that but three-fifths of total solids are directly concerned.

Sulphur compounds

By estimating sulphur in the various fractions we obtain information about the relative amounts of several important brain compounds. The lipin sulphur is a measure of the amount of sulphatid, and is therefore largely concerned in sheath development. In consequence it obtains a maximum rate of formation at about three months of age. Protein sulphur represents the amount of cystin in protein combination. Cystin is present in much smaller amounts in the nucleoproteins of the brain than in neurokeratin. So protein sulphur gives us a rough estimate of the amounts of neurokeratin being formed. It will be noticed that this form of sulphur follows very closely myelin formation (table 10). Neutral sulphur may represent an intermediate oxidation product of cystin or possibly taurin; an increase in this form might indicate a decreased oxidative ability in the cells (W. and M. L. Koch, '13). Of the total sulphur, neutral sulphur forms a greater portion in the younger tissue, while the portion of protein sulphur increases with age. The inorganic sulphates are the final sulphur oxidation products. They remain rather constant in percentage amounts (table 11). This may be due to the fact that they are readily eliminated from the cell. Total sulphur increases in percentage of fresh tissue until adult age, then remains rather constant. The maximum addition, of about 3 mg. per day, takes place at about three months of age (table 13).

Phosphorus compounds

The amount of phosphorus in the lipins is used to determine the amount of the phospho-lipins. It is added most rapidly at birth (table 13, fig. 2) (at least 3.5 mg. per day), but like most of the other constituents, its rate of addition per unit of reaction substances is greatest in the youngest tissue (tables 14 and 15, fig. 3).

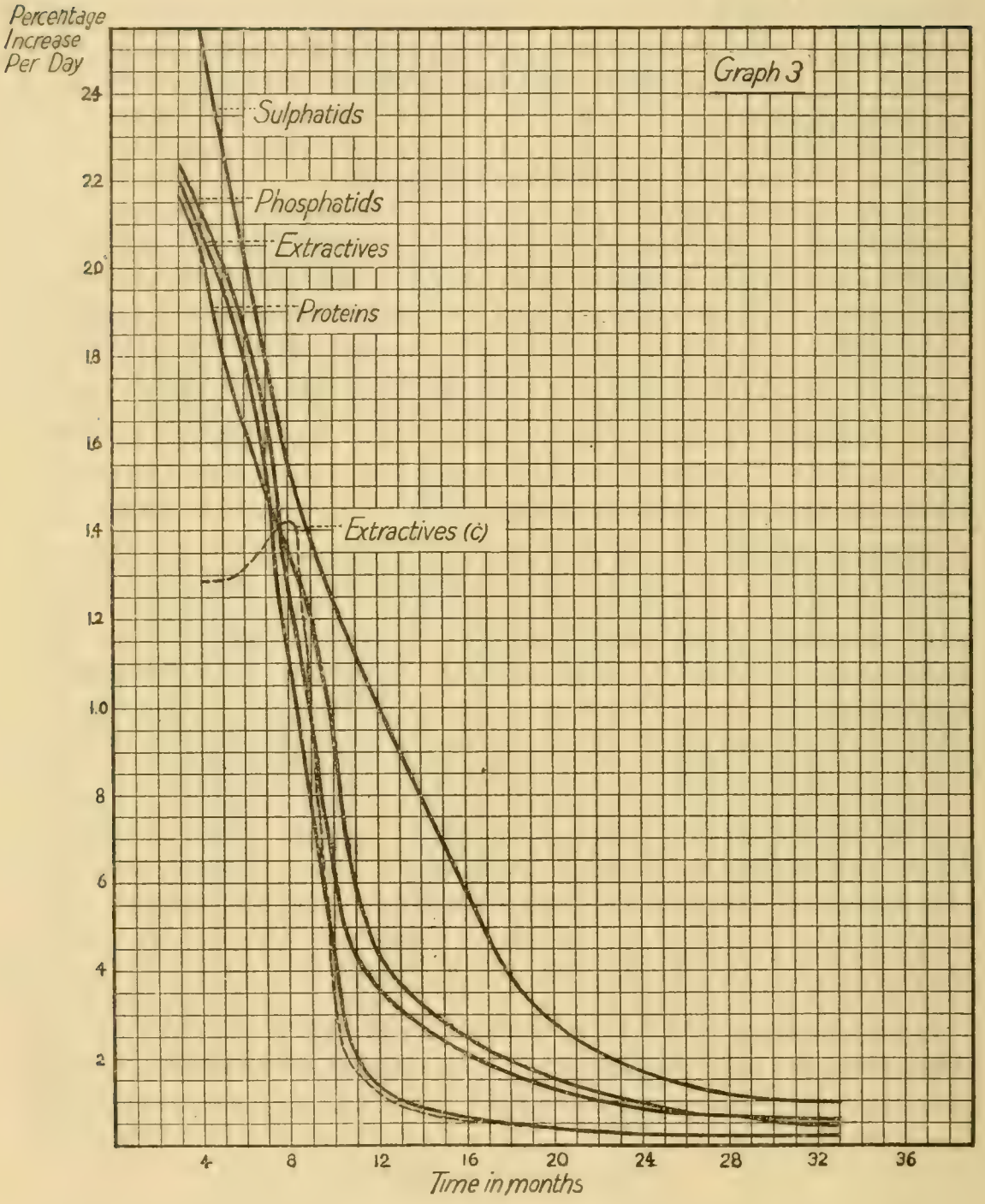


Fig. 3

Protein phosphorus represents the amount of nucleoprotein. This probably closely approximates the changes in the activity of the protoplasm, both nucleus and cytoplasm. This form increases in amount with age, but the percentage changes less than any other kind.

Under the heading of organic phosphorus is included a number of comparatively simple compounds of phosphorus with organic radicles. There is no definite separation of this group from the following one (Emmett and Grindley, '06). It is representative of the amount of protein metabolism and bears a definite relation to the two colloidal forms of phosphorus mentioned above.

Inorganic phosphates are also a measure of rate of activity. In fact, the sum of these last two forms the best criterion of rate of phosphorus metabolism. It is worth noticing that in terms of percentage of total phosphorus the amounts of colloidal phosphorus compounds are increasing with growth, while those of the crystalloidal forms are decreasing (table 11). In fresh tissue the percentage of extractive phosphorus increases slightly, then decreases to a certain extent; but these small variations from a constant may not be significant. The figures, however, indicate rather definitely that these simpler forms of phosphorus do not closely follow the change in percentage of water, as one would expect if the quantity of fluid determined the amount of extractives. From table 11 it is evident that extractive phosphorus, like other extractives, markedly decreases in the percentage of total solids.

Comparison of forebrain, cerebellum, and brain stem

In the adult brain the cerebellum contains the largest percentage of water, the forebrain slightly less, the brain stem least. A high percentage of solids indicates slower but more highly dfferentiated metabolism; therefore the cerebellum acts fastest, while the brain stem is most stereotyped. During growth the rate of increase in the percentage of solids is in the following order, brain stem (table 4), forebrain (table 1), and cerebellum (table 7); this is, of course, to be expected.

In the stem the phosphatids are in larger amounts (table 5) and are probably laid down earlier than in the other two divisions of the brain. The cerebellum contains the smallest amount of this group of constituents (table 8), indicating that it is probably less highly specialized than other parts.

The cerebrosides and sulphatids are present in slightly larger amounts in the stem (table 5) than in the forebrain (table 2), but in very much larger quantities than in the cerebellum (table 8). This would indicate that one of the main differences in chemical constitution between the cerebellum and the rest of the brain is in the amount of medullation.

Cholesterol, etc., is found most largely in the stem and least in the cerebellum.

The total lipins are not only largest in amount in the stem (table 5) and least in the cerebellum (table 8), but possibly are formed slightly earlier and at a more rapid rate in the same order.

In all divisions the proteins show in general variations exactly opposite to that of the lipins. Thus with age there is a decrease in percentage of solids (tables 2, 5, and 8). The total proteins exist in but slightly different percentages in the different parts of the fresh tissue (tables 1, 4, and 7), and they seem to be formed at approximately the same rate in all.

In an attempt further to analyze the meaning of these variations in protein content, the data from protein sulphur and protein phosphorus are of value. It is probable that the greater amount of protein sulphur is in neurokeratin, a constituent of supporting tissue, while the protein phosphorus is very largely present as nucleoprotein *b* (0.6 per cent P), only a relatively small amount being present as nucleoprotein *a* (0.11 per cent P). (The percentage amount of the former, 10 per cent, is about twice that of the latter, 5 per cent in adult tissue.) From tables 1, 4, and 7, then, it will be noticed that there is more than twice as much nucleoprotein in the cerebellum as in other parts of the brain. This difference prevails throughout growth. The stem and forebrain differ but little in this respect. On the other hand, protein sulphur (neurokeratin) is a little greater in the brain stem than in the forebrain or cerebellum. To judge from these data, one might state that the number of nuclei, or at least the amount of nuclear material, is considerably larger in the cerebellum, while the amount of supporting tissue is not very different in the

various parts of the brain. Concerning the extra nuclear proteins (nucleoprotein *a*) it is difficult to make more than a guess—that they would vary with the other functioning protein (nucleoprotein *b*).

Neutral sulphur is a rough measure of the amount of protein metabolism taking place. It is rather striking that the greatest rate of protein metabolism is in the forebrain (table 2) and cerebellum (table 8) and least in the brain stem (table 5). The rate in all remains rather constant in spite of the fact that the amount of protein increases with age.

Throughout growth the total extractives are present in much larger amounts in the cerebellum (table 8) than in the rest of the brain. The stem has a slightly larger percentage than the forebrain. In all divisions the maximum addition per day is reached at about three months of age (tables 2, 5, and 8, fig. 2). After this there is a slow decrease in amount of daily additions till old age, indicating that aging is a regular decrease in rate of metabolism. The larger part of the extractives in the cerebellum is composed of inorganic constituents. This is probably to be expected from the larger protein content in this division of the brain.

The total sulphur increases more rapidly and attains a somewhat greater percentage in the brain stem than in the forebrain (table 1) and a considerably greater percentage than in the cerebellum (table 7). This seems to be largely due to the relatively larger amounts of lipins in each division in the order named. The inorganic sulphates gradually increase in each division at about the same rate.

The amount of total phosphorus does not differ much in different parts of the brain, but the lipin phosphorus is greater in the stem (table 5) and forebrain (table 2) than in the cerebellum (table 8). The inorganic phosphates seem to be closely related to nucleoprotein *b*, because they are represented to a much greater extent in the cerebellum than in the other divisions of the brain.

GENERAL DISCUSSION

1. Dominance of the nervous system

There seem to be no facts presented in this paper that are inconsistent with the theory that the chromosomes are very important in the early differentiation of cells. In fact, the data suggest that nucleoproteins (tables 10 and 11, fig. 1) then phosphatids and simple extractive molecules dominate in the youngest tissue. It is probable that there is a metabolic gradient in the fertilized cell that is important in determining which will be the head end of the organism (Child, '12). Very early in growth nervous tissue differentiates in this head region. Because of this early formation, much of the later development in other parts of the body is rather dependent on the nervous system. It needs to be emphasized that this dominance of one substance over another (or one organ over another) is but relative. Most of them are developing together, but their influence on each other is very different. Very probably growth consists in the formation of continually larger quantities of the respective cell constituents in a more or less definite order, commencing with the nucleoproteins (table 11, fig. 2) of the chromosomes. In the brain there is rapid formation of certain substances, then the presence and formation of the substances influence the rate of formation of another substance, this another, and so on till all have come into adjustment with the new conditions. While these changes are occurring in the brain, and partly because of them, other areas of differentiation are split off, which are to become other organs. Then in these areas a similar cycle of changes will occur. Probably each of the organs has periods of maximum growth. When such unusually large amounts of material are being formed rapidly, in comparison with the rate of formation at other periods of growth, we get an irregularity in the main curve of growth that produces a so-called growth cycle.

During growth substances are regularly coming to the various organs through the blood. We do not now believe that the amount of either these food substances or the oxygen determines the rate of growth, though they do influence it. The

cells in any organ seem to grow somewhat in unison, but they are influenced by cells of other organs, both through the blood and through the nerves. There is no doubt that each organ directly influences the growth of every other in both of these ways. Certain glands secrete substances and discharge them into the circulation that have a disproportionately large effect in influencing growth in most tissues. But it is not correct to assume that growth is determined by these; it is only altered by them. Growth seems to be a general cell process, and these substances simply change the rate of this general cell development.

The growth of the brain is probably less under the influence of internal secretions than other organs. Indeed, there is strong evidence that the secretions are largely under the influence of the nervous system. There is no indication of internal secretions in simple organisms, yet these organisms have a similar form of growth.

2. Relation of these data to four physiological facts

In any interpretation of brain growth it is necessary to keep in mind several important facts:

1. A larger amount of early differentiation occurs in nervous tissue than in other tissues. Though this fact is associated with some definite differences that exist in the fertilized cell, the subsequent chemical supremacy is important in evolving the marked specialization. The early formation of colloidal substances such as the nucleoproteins, phosphatids, and sulphatids (table 10) give a peculiarity to young nerve tissue that permits it to influence rather markedly the development of other tissues. It is more than theoretical to assume that the early start of nervous tissue allows it to differentiate more than other parts of the body.

2. The nerve cells, unlike other cells, do not regenerate. It is very probable that a nerve cell if tested for regenerative power early enough in its growth would regenerate just as other undifferentiated tissues do. It very soon reaches a stage, however, when it is so highly specialized by the elaboration of colloidal complexes (table 11) that regeneration is impossible.

3. The number of nerve cells remains constant. Rather early in growth, probably as early as the seventh fetal month (table 10) the number of nerve cells is largely determined. No amount of functioning produces an increase. This would indicate that the chemical changes in brain growth are fixed within rather close limits. It also suggests that development in the brain is essentially different than elsewhere. Probably the main processes are determined at the time of the formation of the cells. The organization is such, however, that smaller but no less important (speaking physiologically) change occurs during later activity.

4. Nervous tissues remain constant in composition under conditions that markedly alter many other tissues. The chemical composition of the nervous system must be related to this supremacy. The large amounts of several of the lipins (table 10) seem to be of importance. Though the large amount of colloidal material in the form of lipins and proteins is often supposed to be indicative of slower metabolism and a lack of dominance (Child, '11), the chemical condition in the brain would suggest that colloidal structure is equally important with the rate of metabolism in maintaining dominance. It is conceivable that in the case of the brain its early importance, due to the high rate of metabolism, should be maintained through specialized activity, even when this rate is no longer greater than the rate in other tissues. The highly specialized nerve fiber and cell are made of many compounds that are but slightly available to other tissues, because such substances are in almost irreversible equilibrium with metabolizing substances elsewhere.

Another factor that is undoubtedly involved is the selective nature of the membranes surrounding the cells of the nervous system. Very probably such membranes or surfaces are much more common than is supposed, thus providing means of keeping the various tissues in equilibrium with each other. If the membranes in the nervous system are more nearly irreversible than in other parts, the condition exists that is favorable for maintenance under circumstances that use up other tissues.

No other tissue has a chance to supplant the nervous system with its highly specialized pathways to all parts of the body.

It does not need to depend on its rate of activity for supremacy; the conditions it has developed for its maintenance assure this dominance though the means used for obtaining it no longer exist.

3. Concerning three periods of growth

There are three distinct processes to be distinguished in brain development. The one that takes place first is cell division. This is probably almost completed at the time of the seven-month-old fetus. It is worth noting that there is no evidence of sheath development up to this point. There are no cerebrosides; the amounts of sulphatids are increasing slowly. The phosphatids do not show any dominance. The relatively large amounts of protein, and especially the nucleoproteins, suggest that chromosome formation is very prominent. The large quantity of extractives emphasizes the fact that metabolism is very rapid during this period.

From the seven-month fetus to about the time of birth, cell growth is the important process. At this time the phosphatids come into prominence, while the proteins and extractives retain their earlier dominance. Cholesterol, though present, is not important. The same is true of sulphatids, while the cerebrosides are lacking entirely or are present in but small amounts. These changes are what one would expect in growing cells and enlarging axis cylinders. How important the axis cylinders are in accounting for brain growth is indicated by the fact that about two-fifths of the brain consists of them.

The third period is that of medullation. It becomes prominent soon after birth, reaches its maximum a few months after birth, and slowly decreases in importance. The sheaths comprise about two-fifths of the brain substance, so it is not surprising that cerebrosides, sulphatids, and some of the phosphatids become so prominent. The proteins and extractives are still of importance, but thoroughly masked by the new process. It is probable that when the nerve cells reach the stage at which conditions are proper for sheath formation, there is a release of energy or an alteration in metabolism through the extension of

the field of local dominance that is large enough to amount to a slowing down temporarily of the rate of loss of growth power.

By comparing these results with those obtained in a growth series on the brain of the albino rat (W. and M. L. Koch, '13) a great similarity is evident. The nature of the process, the division into periods, the relative amounts of the various constituents, and their order of development are much alike. The main differences are found in the larger percentage amounts of lipins, with a corresponding decrease in proteins and a great lengthening of the periods of growth. Thus the changes occurring in the rat brain are much more rapid than in human brain, but the rat brain does not attain quite the same degree of differentiation. By comparing the data in the two series for extractives as a whole, and the various extractives, no marked differences are evident, indicating that the changes in rate of metabolism with growth are similar in both, though the time necessary to change from one corresponding physiological age to another in the rat is probably but about one-thirtieth of that in the human.

4. Nature of the growth process

One of the most interesting points which these data raise is that of the nature of the growth process in nervous tissue. The curves show that the brain as a whole, as well as each of the individual substances or groups of substances (tables 12 and 13, figs. 1 and 2) estimated, increases slowly in absolute amounts per unit of time during the first part of development. Later the increase is larger, and is then followed by a period when the amounts are continually smaller. If, however, one observes the curve for the amount added per unit mass of substance during a given period of time (tables 14 and 15, fig. 3) it is seen that the rate of addition is greatest in the youngest tissue. This rate of addition diminishes most at first, then more slowly, and is followed by a somewhat greater comparative rate of loss of growth power. The first curves (absolute amounts) are smilar to those reported for growth of the whole organism (Robertson, '08). Such curves are by some authors supposed to indicate that the process they

represent is an autocatalytic one (a chemical reaction that increases in speed at first because of the catalyzing effect of a product of the reaction, and then slows down, because of the retarding effect of larger amounts of a product of the reaction and decrease in the original substance). Can the second curve, however, be reconciled with this theory? From this curve it would seem that the rate of reaction is fastest at first and slows down continuously during growth (Meyer, '14).

There is no inconsistency between these two facts (1st, that the absolute amount of substance added is greatest during the middle period of development; 2d, that the amount of substance added per unit mass is greatest at an early period of development) if we make certain assumptions. It is necessary to assume that all or nearly all of the substance (or group of substances, or total brain, or total organism) is a product of this reaction or determined by some other reaction. The substances weighed are entirely (within limits of error in data) the product of something not weighed or too small to make a significant difference in the weighing. This means that the cytoplasm, and probably nucleus (Loeb, '06), is a product of something else either present and very small, or absent, or not weighable. The easiest interpretation of this difficulty is to invoke the aid of vitalism. This would furnish our unweighable element that determines the growth of even the protoplasm. However, if something a little more substantial is required, one can assume the presence in the brain (or in some other part of the body connected physiologically with the brain) of a very small amount of a substance that in some way determines the formation of all other substances in the tissue (or organism) considered. This substance probably would decrease during growth. One or more of its products would catalyze its effect on formation of other substances. It might exert its control over other reactions by operating over a longer period of time or by having an unusual nature. It is conceivable that a hormone or enzyme-like compound might have such unlimited power. This would assume that at fertilization, or soon after, this substance was made, and that subsequent development is essentially a product of it. Aging would mean the using

up of this substance or an interference with its rate of reaction. Any variations in growth would be due to alterations in the general growth produced by other substances or conditions.

Though the hormones and the active principles in the internal secretions are very popular these days, it seems rather too much to expect that one and only one of them possesses such vitalistic properties. It seems more rational to suppose that they are active in bringing about alterations in growth, but that the main process is independent of them. There is practically no evidence that such substances are determiners of growth in unicellular organisms. If one accepts the autocatalytic theory, it seems necessary to give up the protoplasmic theory, for protoplasm, too, should be simply a product and does not possess growing power. As a result of this and other work, it can be stated with considerable certainty that neither nucleus nor cytoplasm causes growth to take place autocatalytically. If one believes that the evidence for the living, growing nature of the protoplasm as a whole is well founded, chemical autocatalysis should be discarded. The data agree so well with the theory, however, that there must be some reason why a substance in a living organism, as well as the whole tissue or organism should add largest absolute amounts of substance (table 13, fig. 2) during the middle of the growth period. It is worthy of mention, though it is probably not a fundamental explanation to say that protoplasm has an inherent power, when unimpeded by the lack of food or too much of the products of its activity, to increase in a geometrical ratio. As is well known, bacteria and unicellular organisms increase in number and in absolute weight (when retarding factors are small) in this 1, 2, 4, 8, 16 ratio. If such numbers are plotted against time, the first part of the S-shaped curve is obtained. The latter half of the curve is produced through decreasing the geometrical ratio by the retarding effect of lack of food or production of toxic products. By analogy, such a curve should be produced in a multicellular organism, through the division and development of the cells producing it. It is thus seen that the essential characteristics of autocatalysis are the necessary result of cell division in an imperfect environment. Of course, one of the reasons why cells do

not divide so often when there are more of them in a more unfavorable medium is that the individual cells do not grow to the dividing stage so quickly. However, one can apply the geometrical ratio idea to the development of the individual cell if that is found to increase in absolute weight fastest during the middle period of growth. In fact, it is rather to be expected that such would be the form of its growth, without its being in any way related to autocatalysis. For if the protoplasm formed on cell division is thought of as a unit of protoplasm, it would form two units in a certain period of time; then these two would form four, and so on through the geometrical series, if no retarding factors were present. But there are undoubtedly such factors, so we get essentially the autocatalytic phenomenon. The size of the cell, the relation of size of the nucleus to that of the cytoplasm, the amount of cell differentiation, complexity of colloidal substratum of cell are large factors in determining this form of growth. There seems to be a physiological state that is rather definite for any kind of cell, which, unaltered, tends to make the cells increase. One sees the necessity, granting the power of protoplasm to produce more material like itself, in an increasingly unfavorable environment, for the S-shaped curve of growth. This is independent of the question why growth takes place; it is true, irrespective of the nature of the growth impulse. It is probably not wise, however, to speak of such growth as autocatalytic, because it probably does not have a chemical autocatalytic basis. Though enzymes seem to play a part in it, it is not necessarily enzymic at all, much less autocatalytic and monomolecular. Probably anything that can increase geometrically, put under progressively less favorable conditions, whether living or not (say, the growth of a crystal in a slightly supersaturated solution), would give an autocatalytic form of increase.

SUMMARY

1. During growth the proteins, phosphatids, sulphatids, cerebrosides, cholesterol, and total solids increase in percentage amounts. There is but slight change in the percentage of ex-

tractives, either organic or inorganic. Water decreases regularly to maturity.

2. In percentage of solids each of the lipins increases rapidly until a few months after birth, then more slowly until maturity. (Cerebrosides are not present in free condition till about the time of birth.) The proteins slowly decrease in percentage of solids with growth, but the extractives, both organic and inorganic, very rapidly decrease.

3. At birth most of the brain compounds are being laid down most rapidly. Cerebrosides and sulphatids, however, have the greatest daily additions about three months after birth.

The following amounts, in milligrams, are added per day in a new-born child: water 3270, solids 494, lipins 165, phosphatids 85, cholesterol + 70, sulphatids 7.7, cerebrosides 1.9, proteins 186, organic extractives 100, inorganic extractives 44, sulphur 2.3, phosphorus 8.5.

4. The brain stem contains the largest percentage amounts of total solids, total lipins, and of each lipin, but the least protein, organic extractives, inorganic extractives, and water. The forebrain is not much different from the stem. The cerebellum, however, varies largely. In development, the brain stem differentiates chemically first and fastest. The forebrain follows closely. The cerebellum never attains to such a high degree of specialization.

The data may indicate that the cerebellum is not only the slowest and least medullated, but that it remains the youngest division of the brain with the highest rate of metabolism.

5. It is suggested that, because of the early marked chemical differentiation of the brain in the head end of the organism, further development is greatly influenced by the central nervous system.

6. An attempt is made to correlate the data obtained with the early differentiation of specialized nerve tissue and its constancy in number of cells and composition.

7. The chemical analyses agree that brain growth consists of 1) increase in the number of cells; 2) their growth, including that of the axis cylinders, and 3) medullation.

8. The data show that, though the absolute amount of each of the constituents added is greatest during a middle period of growth (birth), the greatest rate of growth is in the youngest tissue. It is not believed that brain growth is necessarily autocatalytic. The whole brain, as well as each constituent, increases with development, as is to be expected if it is assumed that a given mass of protoplasm makes more material like itself in an increasingly less favorable environment. It seems to be a logical necessity, not even dependent upon life.

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TABLE 1

Forebrain: Constituents in percentage of fresh tissue

	FETUS 3 MONTHS (13)[1]	FETUS 7 MONTHS (12)[2]	CHILD 1 MONTH (11)	CHILD 3 MONTHS (7)	CHILD 8 MONTHS (4)[3]	ADULT 21 YEARS (23)	ADULT (33) YEARS (28)	ADULT 35 YEARS (3)	ADULT 67 YEARS (22)
Water	91.91	90.56	88.09	87.03	85.81	77.32	77.06	72.85	78.47
Solids	8.09	9.44	11.91	12.97	14.19	22.68	22.94	27.15	21.53
Phosphatids	1.04	1.24	1.94	(1.74)[4]	3.17	5.68	6.00	6.86	6.54
Cerebrosides	0	0	0	.30	0.49	1.29	1.28	2.58	1.72
Sulphatids	0.16	0.27	0.25	.50	0.50	1.84	0.66	1.72	1.35
Cholesterol	0.58	0.97	1.53	1.70	0.91	3.63	4.81	4.08	2.55
Total lipins	1.78	2.48	3.71	4.24	5.06	12.44	12.75	15.23	12.15
Total proteins	3.77	3.98	4.57	5.29	6.09	8.03	8.11	8.99	7.53
Organic extractives	1.54	1.77	2.44	2.38	2.01	1.19	1.11	2.03	0.88
Inorganic extractives	1.00	1.21	1.19	1.06	1.03	1.02	0.96	0.91	0.96
Total extractives	2.54	2.98	3.63	3.44	3.04	2.21	2.07	2.94	1.84
Lipin sulphur	0.003	0.005	0.005	0.010	0.010	0.036	0.013	0.034	0.027
Protein sulphur	0.026	0.028	0.033	0.038	0.069	0.053	0.052	0.039	0.061
Neutral sulphur	0.015	0.018	0.022	0.026	0.018	0.013	0.007	0.022	0.015
Inorganic sulphur	0.001	0.002	0.004	0.005	0.012	0.002	0.003	0.009	0.003
Total sulphur	0.045	0.053	0.064	0.079	0.109	0.104	0.075	0.104	0.106
Lipin phosphorus	0.044	0.054	0.080	0.078	0.127	0.256	0.284	0.300	0.254
Protein phosphorus	0.025	0.009	0.005	0.006	0.008	0.013	0.011	0.014	0.012
Organic phosphorus	0.026	0.028	0.036	0.055	0.032	0.012	0.027	0.049	0.008
Inorganic phosphorus	0.056	0.062	0.082	0.074	0.055	0.058	0.091	0.048	0.053
Total phosphorus	0.151	0.153	0.203	0.213	0.222	0.339	0.363	0.411	0.327

[1] The two brains of this age that were used for this analysis were not separated into cerebellum, forebrain, and stem, because the brains were too small to make good samples of these divisions. For purposes of comparison the whole brain was arbitrarily divided into cerebellum 10 per cent, brain stem 10 per cent, and forebrain 80 per cent.

[2] The same is true of this brain.

[3] See section on limitations.

[4] See section on limitations for explanation of this low figure.

TABLE 2

Forebrain: Constituents in percentage of solids

	FETUS 3 MONTHS (13)[1]	FETUS 7 MONTHS (12)[1]	CHILD 1 MONTH (11)	CHILD 3 MONTHS (7)	CHILD 8 MONTHS (4)[1]	ADULT 21 YEARS (23)	ADULT 33 YEARS (28)	ADULT 35 YEARS (3)	ADULT 35 YEARS (19)	ADULT 67 YEARS (22)
Phosphatids..	12.90	13.12	16.27	(13.40)[1]	22.33	25.06	24.67	25.26	25.34	27.19
Cerebrosides..	0	0	0	2.32	3.43	5.67	5.59	9.50	7.13	8.00
Sulphatids....	1.95	2.85	2.06	3.87	3.53	8.12	2.89	6.32	6.90	6.26
Cholesterol...	7.24	10.28	12.86	13.09	6.39	16.02	22.45	15.01	18.47	15.03
Total lipins.....	22.09	26.25	31.19	32.68	35.68	54.87	55.60	56.09	57.84	56.48
Total proteins..	46.56	42.15	38.31	40.78	42.91	35.40	35.36	33.10	32.27	34.97
Organic extractives....	19.04	18.75	20.52	18.35	14.18	5.23	4.85	7.46	6.12	4.08
Inorganic extractives....	12.31	12.85	9.98	8.19	7.23	4.50	4.19	3.35	3.77	4.47
Total extractives.....	31.35	31.60	30.50	26.54	21.41	9.73	9.04	10.81	9.89	8.55
Lipin sulphur.	0.039	0.057	0.041	0.077	0.071	0.163	0.058	0.127	0.138	0.125
Protein sulphur.....	0.314	0.294	0.277	0.287	0.483	0.235	0.225	0.146	0.279	0.279
Neutral sulphur.....	0.187	0.183	0.190	0.195	0.128	0.057	0.029	0.081	0.022	0.070
Inorganic sulphur.....	0.013	0.022	0.038	0.037	0.085	0.011	0.015	0.032	0.029	0.015
Total sulphur...	0.553	0.556	0.546	0.596	0.767	0.466	0.327	0.386	0.468	0.489
Lipin phosphorus.....	0.538	0.563	0.676	0.597	0.886	1.136	1.017	1.110	1.120	1.179
Protein phosphorus.....	0.306	0.100	0.041	0.045	0.056	0.055	0.048	0.052	0.063	0.057
Organic phosphorus.....	0.322	0.300	0.306	0.422	0.225	0.053	0.117	0.180	0.226	0.035
Inorganic phosphorus.	0.678	0.648	0.693	0.565	0.384	0.258	0.394	0.172	0.189	0.244
Total phosphorus.....	1.844	1.611	1.716	1.629	1.551	1.502	1.576	1.515	1.598	1.515

[1] See table 1.

TABLE 3

Forebrain: Weights of constituents in grams

	FETUS 3 MONTHS (13)[1]	FETUS 7 MONTHS (12)[1]	CHILD 1 MONTH (11)	CHILD 3 MONTHS (7)	CHILD 8 MONTHS (4)[1]	ADULT 21 YEARS (23)	ADULT 33 YEARS (28)	ADULT 35 YEARS (3)	ADULT 67 YEARS (22)
Brain.....	17.08	119.0	457.4	585.2	492.5	1122.4	1221.3	1158.3	1297.9
Forebrain..	13.664	95.2	395.0	514.0	409.0	950.0	1026.0	986.0	1075.0
Water.....	12.56	86.16	347.9	447.3	351.0	734.5	790.7	718.3	843.6
Solids.....	1.104	9.04	47.1	66.68	58.0	215.5	235.3	267.7	231.5
Phosphatids....	0.1424	1.1808	7.660	8.940	12.96	53.95	61.56	67.64	70.33
Cerebrosides...	0.0000	0.0000	0.000	1.542	2.00	12.26	13.13	25.44	18.49
Sulfatids....	0.0216	0.2568	0.980	2.570	2.05	17.48	6.77	16.96	14.52
Cholesterol...	0.0792	0.9232	6.043	8.738	3.72	34.48	49.34	40.24	27.42
Total lipins....	0.2432	2.3608	14.660	21.80	20.69	118.1	130.80	150.28	130.60
Total proteins.....	0.5152	3.7888	18.05	27.19	24.91	76.28	83.20	88.65	80.97
Organic extractives..	0.2104	1.6848	9.638	12.24	8.22	11.31	11.39	20.02	9.46
Inorganic extractives...	0.1368	1.152	4.700	5.45	4.21	9.69	9.85	8.97	10.32
Total extractives	0.3472	2.8368	14.338	17.69	12.43	21.00	21.24	28.99	19.78
Lipin sulphur...	0.0004	0.0048	0.020	0.051	0.041	0.342	0.133	0.335	0.290
Protein sulphur...	0.0035	0.0266	0.130	0.195	0.282	0.504	0.533	0.385	0.656
Neutral sulphur...	0.0021	0.0171	0.187	0.134	0.074	0.123	0.072	0.217	0.161
Inorganic sulphur...	0.0002	0.0019	0.016	0.026	0.048	0.019	0.031	0.088	0.032
Total sulphur.....	0.0062	0.0505	0.253	0.406	0.446	0.988	0.770	1.025	1.140

TABLE 3—*Concluded*

	FETUS 3 MONTHS (13)[1]	FETUS 7 MONTHS (12)[1]	CHILD 1 MONTH (11)	CHILD 3 MONTHS (7)	CHILD 8 MONTHS (4)[1]	ADULT 21 YEARS (23)	ADULT 33 YEARS (28)	ADULT 35 YEARS (3)	ADULT (67) YEARS (22)
Lipin phosphorus	0.0060	0.0514	0.316	0.401	0.519	2.432	2.401	2.958	2.731
Protein phosphorus	0.0034	0.0086	0.020	0.031	0.033	0.124	0.113	0.138	0.129
Organic phosphorus	0.0025	0.0266	0.142	0.283	0.131	0.114	0.277	0.483	0.086
Inorganic phosphorus	0.0077	0.0590	0.324	0.380	0.225	0.551	0.934	0.473	0.570
Total phosphorus	0.0206	0.1457	0.802	1.095	0.908	3.221	3.724	4.052	3.516

[1] See table 1.

TABLE

Brain stem: Constituents in percentages of solids

	FETUS 3 MONTHS (13)[1]	FETUS 7 MONTHS (12)[1]	CHILD 1 MONTH (14)[2]	CHILD 3 MONTHS (20)	CHILD 8 MONTHS (21)[1]	ADULT 21 YEARS (27)	ADULT 35 YEARS (18)	ADULT 67 YEARS (26)
Phosphatids	12.90	13.12	17.29	(25.86)	16.67	22.85	15.73	30.86
Cerebrosides	0.00	0.00	1.95	1.08	2.33	(0.81)	4.58	9.83
Sulfatids	1.95	2.85	0.44	4.48	5.40	7.45	8.40	7.52
Cholesterol	7.24	10.28	16.32	13.38	19.75	28.48	31.86	11.87
Total lipins	22.09	26.25	36.00	44.80	44.15	59.59	60.57	60.08
Total proteins	46.56	42.15	39.21	40.55	40.52	31.41	29.96	32.01
Organic extractives	19.04	18.75	17.31	8.61	9.50	5.45	5.78	4.01
Inorganic extractives	12.31	12.85	7.66	6.04	5.83	3.55	3.69	3.90
Total extractives	31.35	31.60	24.97	14.65	15.33	9.00	9.47	7.91
Lipin sulphur	0.039	0.057	0.009	0.090	0.108	0.148	0.168	0.150
Protein sulphur	0.314	0.294		0.238	0.272	0.264	0.211	0.289
Neutral sulphur	0.187	0.183	0.158	0.069	0.093	0.009	0.023	0.037
Inorganic sulphur	0.013	0.294	0.036	0.013	0.008	0.015	0.018	0.009
Total sulphur	0.553	0.556		0.410	0.481	0.436	0.420	0.485
Lipin phosphorus	0.538	0.563	0.674	1.100	0.753	1.035	0.778	1.343
Protein phosphorus	0.306	0.100	0.138	0.068	0.064	0.041	0.052	0.044
Organic phosphorus	0.322	0.300	0.252	0.119	0.168	0.064	0.184	0.147
Inorganic phosphorus	0.678	0.648	0.595	0.435	0.425	0.314	0.199	0.238
Total phosphorus	1.844	1.611	1.659	1.722	1.410	1.454	1.213	1.772

[1] See table 1. [2] See table 4.

TABLE 4

Brain stem: Constituents in percentage of fresh tissue

	FETUS 3 MONTHS (13)[1]	FETUS 7 MONTHS (12)[1]	CHILD 1 MONTH (14)[2]	CHILD 3 MONTHS (20)	CHILD 8 MONTHS (21)[1]	ADULT 21 YEARS (27)	ADULT 35 YEARS (18)	ADULT 67 YEARS (26)
Water	91.91	90.56	86.15	84.24	82.71	73.60	70.34	76.26
Solids	8.09	9.44	13.85	15.76	17.29	26.40	29.66	23.74
Phosphatids	1.04	1.24	2.40	(4.07)	2.89	6.03	4.69	7.33
Cerebrosides	0.00	0.00	0.27	0.17	0.40	(0.21)	1.36	2.33
Sulfatids	0.16	0.27	0.06	0.71	0.94	1.97	2.49	1.78
Cholesterol	0.58	0.97	2.26	2.11	3.41	7.52	9.43	2.83
Total lipins	1.78	2.48	4.99	7.06	7.65	15.73	17.97	14.27
Total proteins	3.77	3.98	5.43	6.40	7.01	8.29	8.90	7.60
Organic extractives	1.54	1.77	2.37	1.35	1.64	1.44	1.71	0.95
Inorganic extractives	1.00	1.21	1.06	0.95	1.00	0.94	1.10	0.93
Total extractives	2.54	2.98	3.43	2.30	2.64	2.38	2.81	1.88
Lipin sulphur	0.003	0.005	0.001	0.014	0.019	0.039	0.050	0.036
Protein sulphur	0.026	0.028		0.037	0.047	0.069	0.062	0.069
Neutral sulphur	0.015	0.018	0.022	0.011	0.016	0.002	0.007	0.009
Inorganic sulphur	0.001	0.002	0.005	0.002	0.002	0.004	0.005	0.002
Total sulphur	0.045	0.053		0.064	0.084	0.114	0.124	0.116
Lipin phosphorus	0.044	0.054	0.094	0.172	0.131	0.273	0.231	0.317
Protein phosphorus	0.025	0.009	0.019	0.011	0.011	0.011	0.015	0.011
Organic phosphorus	0.026	0.028	0.035	0.019	0.029	0.017	0.055	0.035
Inorganic phosphorus	0.056	0.062	0.083	0.068	0.074	0.083	0.059	0.058
Total phosphorus	0.151	0.153	0.231	0.270	0.245	0.384	0.360	0.421

[1] See table 1.

[2] Brain stem and cerebellum analyzed together, because of small sample.

TABLE 6

Brain stem: Weight of constituents in grams

	FETUS 3 MONTHS (13)[1]	FETUS 7 MONTHS (12)[1]	CHILD 1 MONTH (14)[2]	CHILD 3 MONTHS (20)	CHILD 8 MONTHS (20)[1]	ADULT 21 YEARS (21)	ADULT 35 YEARS (18)	ADULT 67 YEARS (26)
Brain.....	17.08	119.0	457.4	585.2	492.5	1122.4	1158.3	1297.9
Brain stem.	1.708	11.9	25.0	28.5	35.3	61.0	61.5	77.5
Water.....	1.57	10.77	21.54	24.00	29.20	44.90	43.26	59.10
Solids.....	0.138	1.125	3.46	4.50	6.10	16.10	18.24	18.40
Phosphatids....	0.0178	0.1476	0.600	1.160	1.024	3.678	2.884	5.681
Cerebrosides...	0.0000	0.0000	0.068	0.048	0.141	0.128	0.836	1.806
Sulphatids	0.0027	0.0321	0.015	0.202	0.332	1.202	1.531	1.380
Cholesterol...	0.0099	0.1154	0.565	0.601	1.204	4.587	5.799	2.193
Total lipins.	0.0304	0.2951	1.248	2.012	2.700	9.595	11.052	11.059
Total proteins.....	0.0644	0.4736	1.358	1.814	2.475	5.057	5.474	5.890
Organic extractives....	0.0263	0.2106	0.592	0.385	0.579	0.878	1.052	0.736
Inorganic extractives....	0.0171	0.144	0.265	0.271	0.353	0.573	0.677	0.721
Total extractives..	0.0434	0.3546	0.857	0.656	0.932	1.451	1.729	1.457
Lipin sulphur....	0.0001	0.0006	0.0003	0.0040	0.0138	0.0238	0.0308	0.0279
Protein sulphur.	0.0004	0.0033		0.0105	0.0244	0.0421	0.0381	0.0535
Neutral sulphur.	0.0003	0.0021	0.0055	0.0031	0.0007	0.0012	0.0043	0.0070
Inorganic sulphur.	0.0000	0.0002	0.0013	0.0006	0.0014	0.0024	0.0031	0.0016
Total sulphur.....	0.0008	0.0063		0.0182	0.0402	0.0695	0.0763	0.0900

TABLE 6—*Continued*

	FETUS 3 MONTHS (13)[1]	FETUS 7 MONTHS (12)[1]	CHILD 1 MONTH (14)[2]	CHILD 3 MONTHS (20)	CHILD 8 MONTHS (21)[1]	ADULT 21 YEARS (27)	ADULT 35 YEARS (18)	ADULT 67 YEARS (24)
Lipin phosphorus..	0.0008	0.0064	0.0235	0.0490	0.0462	0.1665	0.1421	0.2457
Protein phosphorus..	0.0004	0.0011	0.0048	0.0031	0.0039	0.0067	0.0092	0.0085
Organic phosphorus..	0.0004	0.0033	0.0088	0.0054	0.0102	0.0104	0.0338	0.0271
Inorganic phosphorus..	0.0010	0.0074	0.0207	0.0194	0.0261	0.0506	0.0363	0.0450
Total phosphorus...	0.0026	0.0182	0.0578	0.0769	0.0865	0.2342	0.2214	0.3263

[1] See table 1.
[2] See table 4.

TABLE 7

Cerebellum. Constituents in percentage of fresh tissue

	FETUS 3 MONTHS (12)[1]	FETUS 7 MONTHS (12)[1]	CHILD 1 MONTH (14)[2]	CHILD 3 MONTHS (17)	CHILD 8 MONTHS (1)[1]	ADULT 1 YEAR (25)	ADULT 35 YEARS (10)	ADULT 67 YEARS (29)
Water	91.91	90.56	86.15	85.05	84.56	78.83	77.99	80.64
Solids	8.09	9.44	13.85	14.95	15.44	21.17	22.01	19.36
Phosphatids	1.04	1.24	2.40	2.70	2.58	6.66	(2.84)	4.07
Cerebrosides	0.00	0.00	(0.27)[3]	0.00	0.26	0.98	0.84	0.54
Sulphatids	0.16	0.27	(0.06)[3]	0.86	0.75	0.94	1.02	0.96
Cholesterol	0.58	0.97	2.26	1.33	1.54	0.89	4.12	3.10
Total lipins	1.78	2.48	4.99	4.89	5.13	9.46	8.82	8.67
Total proteins	3.77	3.98	5.43	6.97	6.94	8.95	8.60	7.66
Organic extractives	1.54	1.77	2.37	1.90	2.10	1.55	(2.97)	1.68
Inorganic extractives	1.00	1.21	1.06	1.19	1.28	1.23	1.61	1.36
Total extractives	2.54	2.98	3.43	3.09	3.38	2.78	(4.58)	3.04
Lipin sulphur	0.003	0.005	0.001	0.018	0.015	0.019	0.020	0.019
Protein sulphur	0.026	0.028		0.041	0.051	0.053	0.067	0.058
Neutral sulphur	0.015	0.018	0.022	0.020	0.014	0.014	0.037	0.006
Inorganic sulphur	0.001	0.002	0.005	0.001	0.002	0.002	0.009	0.002
Total sulphur	0.045	0.053		0.080	0.082	0.088	0.133	0.085
Lipin phosphorus	0.044	0.054	0.094	0.122	0.115	0.278	0.140	0.178
Protein phosphorus	0.025	0.009	0.019	0.045	0.046	0.042	0.036	0.028
Organic phosphorus	0.026	0.028	0.035	0.020	0.039	0.022	0.080	(0.009)
Inorganic phosphorus	0.056	0.062	0.083	0.075	0.086	0.110	0.114	0.106
Total phosphorus	0.151	0.153	0.231	0.262	0.286	0.452	0.370	0.321

[1] See table 1.

[2] See table 4.

[3] Earlier in the paper it was stated, that sulphur determinations were occasionally low. This is the case here. Because of the sugar content of sulphatids, if the latter is too low, these may be reported for cerebrosides when there are none free.

TABLE 8

Cerebellum: Constituents in percentage of solids

	FETUS 3 MONTHS (13)[1]	FETUS 7 MONTHS (12)[1]	CHILD 1 MONTH (14)[2]	CHILD 3 MONTHS (17)	CHILD 8 MONTHS (16)[1]	ADULT 21 YEARS (25)	ADULT 35 YEARS (10)	ADULT 67 YEARS (29)
Phosphatids	12.90	13.12	17.29	18.03	16.71	31.42	12.90	21.00
Cerebrosides	0.00	0.00	(1.95)[3]	0.00	1.65	4.64	3.81	2.77
Sulphatids	1.95	2.85	(0.44)[3]	5.76	4.85	4.37	4.64	4.98
Cholesterol	7.24	10.28	16.32	8.92	9.99	4.18	18.72	16.05
Total lipins	22.09	26.25	36.00	32.71	33.20	44.61	40.07	44.70
Total proteins	46.56	42.15	39.21	46.63	44.92	42.29	39.12	39.51
Organic extractives	19.04	18.75	17.13	12.71	13.59	7.26	13.51	8.68
Inorganic extractives	12.31	12.85	7.66	7.95	8.29	5.84	7.30	7.01
Total extractives	31.35	31.60	24.79	20.66	21.88	13.10	20.81	15.69
Lipin sulphur	0.039	0.051	0.009	0.115	0.097	0.088	0.093	0.100
Protein sulphur	0.314	0.294		0.273	0.323	0.252	0.307	0.296
Neutral Sulphur	0.187	0.183	0.158	0.129	0.092	0.064	0.168	0.032
Inorganic sulphur	0.013	0.022	0.036	0.009	0.015	0.007	0.044	0.009
Total sulphur	0.553	0.556		0.526	0.527	0.411	0.612	0.437
Lipin phosphorus	0.538	0.563	0.674	0.812	0.741	1.310	0.642	0.912
Protein phosphorus	0.306	0.100	0.138	0.302	0.297	0.198	0.163	0.146
Organic phosphorus	0.322	0.300	0.252	0.130	0.249	0.104	0.364	0.046
Inorganic phosphorus	0.678	0.648	0.595	0.502	0.554	0.519	0.522	0.546
Total phosphorus	1.844	1.611	1.659	1.746	1.841	2.131	1.691	1.650

[1] See table 1.
[2] See table 4.
[3] See table 7.

TABLE 9

Cerebellum: Weights of constituents in grams

	FETUS 3 MONTHS (13)[1]	FETUS 7 MONTHS (12)[1]	CHILD 1 MONTH (14)[2]	CHILD 3 MONTHS (17)	CHILD 8 MONTHS (16)[1]	ADULT 21 YEARS (25)	ADULT 35 YEARS (10)	ADULT 67 YEARS (29)
Brain.....	17.08	119.0	457.4	585.2	492.5	1122.4	1158.3	1297.9
Cerebellum.	1.708	11.9	37.4	42.6	48.2	111.4	110.8	145.4
Water.....	1.57	10.77	32.22	36.23	40.76	87.80	86.41	117.25
Solids.....	0.138	1.125	5.18	6.40	7.44	23.58	24.39	28.15
Phosphatids....	0.0178	0.1476	0.898	1.150	1.244	7.418	3.147	5.918
Cerebrosides...	0.0000	0.000	0.101	0.000	0.125	1.091	0.931	0.785
Sulphatids	0.0027	0.0321	0.022	0.366	0.362	1.036	1.130	1.396
Cholesterol...	0.0099	0.1154	0.845	0.567	0.742	0.991	4.565	4.507
Total lipins.	0.0304	0.2951	1.866	2.084	2.473	10.536	9.772	12.606
Total proteins.....	0.0644	0.4736	2.031	2.969	3.345	9.968	9.529	11.138
Organic extractives...	0.0263	0.2106	0.886	0.810	1.012	1.704	3.291	2.443
Inorganic extractives....	0.0171	0.144	0.397	0.507	0.617	1.370	1.784	1.977
Total extractives..	0.0434	0.3546	1.283	1.317	1.629	3.074	5.075	4.420
Lipin sulphur...	0.0001	0.0006	0.0004	0.0077	0.0072	0.0212	0.0222	0.0276
Protein sulphur.	0.0004	0.0033		0.0175	0.0246	0.0590	0.0742	0.0843
Neutral sulphur.	0.0003	0.0021	0.0082	0.0085	0.0067	0.0156	0.0410	0.0087
Inorganic sulphur.	0.0000	0.0002	0.0019	0.0004	0.0010	0.0022	0.0100	0.0029
Total sulphur.....	0.0008	0.0063		0.0341	0.0395	0.0980	0.1474	0.1236

[1] See table 1.
[2] See table 4.

TABLE 9—*Continued*

	FETUS 3 MONTHS (13)[1]	FETUS 9 MONTHS (12)[1]	CHILD 1 MONTH (14)[2]	CHILD 3 MONTHS (17)	CHILD 8 MONTHS (16)[1]	ADULT 21 YEARS (25)	ADULT 35 YEARS (10)	ADULT 67 YEARS (29)
Lipin phosphorus	0.0008	0.0064	0.0352	0.0520	0.0554	0.3097	0.1551	0.2588
Protein phosphorus	0.0004	0.0011	0.0071	0.0192	0.0222	0.0468	0.0399	0.0407
Organic phosphorus	0.0004	0.0033	0.0131	0.0085	0.0188	0.0245	0.0886	0.0131
Inorganic phosphorus	0.0010	0.0074	0.0311	0.0320	0.0415	0.1225	0.1263	0.1541
Total phosphorus	0.0026	0.0182	0.0864	0.1116	0.1378	0.5035	0.4100	0.4667

TABLE 10

Whole brain: Constituents in percentage of fresh tissue

	FETUS 3 MONTHS	FETUS 7 MONTHS	CHILD 1 MONTH	CHILD 3 MONTHS	CHILD 8 MONTHS	ADULT 21 YEARS	ADULT 35 YEARS	ADULT 67 YEARS
Water	91.91	90.56	87.81	86.75	85.47	77.25	73.20	78.58
Solids	8.09	9.44	12.19	13.25	14.53	22.75	26.80	21.42
Phosphatids	1.04	1.24	2.00	1.92	3.09	5.80	6.35	6.30
Cerebrosides	0.00	0.00	.04	.27	.46	1.20	2.35	1.62
Sulphatids	.16	.27	.22	.53	.56	1.75	1.69	1.33
Cholesterol	.58	.97	1.63	1.68	1.15	3.57	4.36	2.62
Total lipins	1.78	2.48	3.89	4.42	5.26	12.32	14.75	11.87
Total proteins	3.77	3.98	4.69	5.47	6.24	8.14	8.95	7.54
Organic extractives	1.54	1.77	2.43	2.29	1.99	1.24	2.10	0.98
Inorganic extractives	1.00	1.21	1.18	1.08	1.05	1.04	0.99	1.01
Total extractives	2.54	2.98	3.61	3.37	3.04	2.28	3.09	1.99
Lipin sulphur	0.003	0.005	0.004	0.011	0.012	0.035	0.034	0.026
Protein sulphur	0.026	0.028	0.029	0.038	0.066	0.054	0.043	0.061
Neutral sulphur	0.015	0.018	0.022	0.026	0.016	0.013	0.023	0.014
Inorganic sulphur	0.001	0.002	0.004	0.005	0.010	0.002	0.009	0.003
Total sulphur	0.045	0.053	0.059	0.080	0.104	0.104	0.109	0.104
Lipin phosphorus	0.044	0.054	0.081	0.086	0.124	0.259	0.280	0.248
Protein phosphorus	0.025	0.009	0.007	0.009	0.012	0.016	0.016	0.014
Organic phosphorus	0.026	0.028	0.035	0.051	0.032	0.013	0.052	0.010
Inorganic phosphorus	0.056	0.062	0.082	0.074	0.060	0.064	0.055	0.059
Total phosphorus	0.151	0.153	0.205	0.220	0.228	0.352	0.403	0.331

TABLE 11

Whole brain: Constituents in percentage of solids

	FETUS 3 MONTHS	FETUS 7 MONTHS	CHILD 1 MONTH	CHILD 3 MONTHS	CHILD 8 MONTHS	ADULT 21 YEARS	ADULT 35 YEARS	ADULT 67 YEARS
Phosphatids	12.90	13.12	16.40	14.50	21.26	25.52	23.69	29.42
Cerebrosides	0.00	0.00	0.33	2.04	3.16	5.28	8.77	7.56
Sulphatids	1.95	2.85	1.80	4.00	3.85	7.70	6.30	6.21
Cholesterol	7.24	10.28	13.37	12.76	7.91	15.70	16.26	12.24
Total lipins	22.09	26.25	31.90	33.30	36.19	54.20	55.01	55.43
Total proteins	46.56	42.15	38.46	41.30	42.93	35.82	33.58	35.21
Organic extractives	19.04	18.75	19.93	17.29	13.69	5.46	7.83	4.58
Inorganic extractives	12.31	12.85	9.68	8.15	7.22	4.58	3.69	4.72
Total extractives	31.35	31.60	29.61	25.44	20.91	10.04	11.52	9.30
Lipin sulphur	0.039	0.057	0.033	0.083	0.083	0.154	0.127	0.121
Protein sulphur	0.314	0.294	0.238	0.287	0.454	0.238	0.160	0.285
Neutral sulphur	0.187	0.183	0.180	0.196	0.110	0.057	0.086	0.065
Inorganic sulphur	0.013	0.022	0.033	0.038	0.068	0.009	0.034	0.014
Total sulphur	0.553	0.556	0.184	0.604	0.715	0.458	0.407	0.485
Lipin phosphorus	0.538	0.563	0.664	0.649	0.853	1.140	1.044	1.158
Protein phosphorus	0.306	0.100	0.057	0.068	0.083	0.070	0.060	0.065
Organic phosphorus	0.322	0.300	0.287	0.385	0.220	0.057	0.194	0.047
Inorganic phosphorus	0.678	0.648	0.672	0.559	0.413	0.282	0.205	0.276
Total phosphorus	1.844	1.611	1.680	1.661	1.569	1.549	1.503	1.546

TABLE 12

Whole brain: Weights of constituents in grams

	FETUS 3 MONTHS	FETUS 7 MONTHS	CHILD 1 MONTH	CHILD 3 MONTHS	CHILD 8 MONTHS	ADULT 21 YEARS	ADULT 35 YEARS	ADULT 67 YEARS
Whole brain	17.08	119.0	457.4	585.2	492.5	1122.4	1158.3	1297.9
Water.....	15.70	107.7	401.7	507.5	421.0	867.2	848.0	1020.0
Solids.....	1.38	11.25	55.74	77.58	71.54	255.2	310.3	278.0
Phosphatids....	0.178	1.476	9.158	11.250	15.228	65.05	73.67	81.93
Cerebrosides...	0.000	0.000	0.169	1.590	2.266	13.479	27.207	21.081
Sulphatids....	0.027	0.321	1.017	3.138	2.744	19.718	19.621	17.296
Cholesterol...	0.099	1.154	7.453	9.906	5.666	40.058	50.604	34.120
Total lipins.	0.304	2.951	17.774	25.896	25.863	138.23	171.02	154.27
Total proteins.....	0.644	4.736	21.439	31.973	30.730	91.305	103.65	98.00
Organic extractives...	0.263	2.106	11.116	13.435	9.811	13.892	24.363	12.639
Inorganic extractives...	0.171	1.440	5.362	6.228	5.180	11.633	11.431	13.018
Total extractives..	0.434	3.546	16.478	19.663	14.991	25.525	35.794	25.657
Lipin sulphur.	0.0005	0.0060	0.0207	0.0627	0.0620	0.3870	0.3880	0.3453
Protein sulphur.	0.0044	0.0333	0.1300	0.2230	0.3310	0.6051	0.4973	0.7938
Neutral sulphur.	0.0026	0.0214	0.1007	0.1456	0.0814	0.1398	0.2623	0.1767
Inorganic sulphur.	0.0002	0.0024	0.0192	0.0270	0.0514	0.0236	0.1011	0.0365
Total sulphur.....	0.0077	0.0631	0.2706	0.4583	0.5248	1.1555	1.2487	1.3525

TABLE 12—*Continued*

	FETUS 3 MONTHS	FETUS 7 MONTHS	CHILD 1 MONTH	CHILD 3 MONTHS	CHILD 8 MONTHS	ADULT 21 YEARS	ADULT 35 YEARS	ADULT 67 YEARS
Lipin phosphorus	0.0075	0.0643	0.3747	0.5020	0.6206	2.0980	3.2550	3.2360
Protein phosphorus	0.0043	0.0102	0.0319	0.0533	0.0591	0.1775	0.1871	0.1782
Organic phosphorus	0.0044	0.0333	0.1639	0.2969	0.1600	0.1489	0.6054	0.1262
Inorganic phosphorus	0.0096	0.0738	0.3758	0.5415	0.2926	0.7241	0.6356	0.7691
Total phosphorus	0.0258	0.1821	0.9463	1.2836	1.1323	3.9585	4.6831	4.3095

Figure 1 was plotted from the data in this table relating to the earlier period of growth.

TABLE 13

Whole brain: Milligrams added per day

	UP TO 3-MONTH FETUS	3-MONTH TO 7-MONTH FETUS	7-MONTH FETUS TO 1-MONTH CHILD	1-MONTH TO 3-MONTH CHILD	3-MONTH TO 8-MONTH CHILD	8-MONTH TO 21-YEAR
Whole brain	190.0	848.0	3764.0	2127.0	501.6	131.2
Water	174.0	766.0	3270.0	1763.0	417.0	113.0
Solids	15.3	82.3	494.0	364.0	84.6	18.2
Phosphatids	1.98	10.80	85.3	34.9	26.3	5.4
Cerebrosides	0.0	0.0	1.88	23.7	4.07	1.4
Sulphatids	0.30	2.45	7.73	35.4	2.99	2.2
Cholesterol	1.10	8.79	70.0	40.9	0.74	4.4
Total lipins	3.38	22.04	164.9	135.1	33.9	13.4
Total proteins	7.16	34.1	185.6	175.6	38.5	5.1
Organic extractives	2.81	15.4	100.1	38.7	7.2	−0.6
Inorganic extractives	1.90	10.6	43.6	14.4	5.2	−0.3
Total extractives	4.81	26.0	143.7	53.1	11.4	−0.3
Lipin sulphur	0.006	0.05	0.16	0.70	0.08	0.04
Protein sulphur	0.049	0.24	1.07	1.55	0.61	0.0
Neutral sulphur	0.029	0.16	0.88	0.75	0.01	0.0
Inorganic sulphur	0.002	0.02	0.19	0.13	0.11	−0.01
Total sulphur	0.086	0.47	2.30	3.13	0.81	0.03
Lipin phosphorus	0.083	0.47	3.45	2.12	1.01	0.27
Protein phosphorus	0.048	0.081	0.24	0.36	0.09	0.01
Organic phosphorus	0.049	0.24	1.45	2.22	0.0	−0.02
Inorganic phosphorus	0.107	0.54	3.36	0.93	0.17	0.03
Total phosphorus	0.287	1.30	8.50	5.62	1.27	0.29

A part of these data are plotted in graph 2.

TABLE 14

Whole brain: Average percentage increase per day

	3-MONTH FETUS	7-MONTH FETUS	1-MONTH	3-MONTH	8-MONTH
Whole brain	2.3	1.7	0.88	0.26	0.028
Water	2.1	1.7	0.88	0.23	0.04
Solids	2.4	1.7	1.0	0.36	0.046
Phosphatids	2.3	1.8	0.79	0.36	0.03
Cerebrosides	0.0	0.0	4.7	1.6	0.057
Sulphatids	2.7	1.9	1.3	1.1	0.072
Cholesterol	2.5	1.8	1.1	0.1	0.02
Total lipins	2.2	1.8	1.3	0.32	0.03
Proteins	2.3	1.6	1.1	0.5	0.02
Organic extractives	2.3	1.7	0.42	0.13	0.00
Inorganic extractives	2.2	1.6	0.56	0.26	0.00
Total extractives	2.3	1.7	0.6	0.15	0.00

These data were estimated from curves similar to, but larger than curves (1). These were plotted from data in table 10.

In the calculation from the curves a period of one-half month before and one-half month after each age was used. It is believed that the enormous figures for rate of growth sometimes presented for early fetal life are due to the method of calculating from the weight at the beginning of the period. When the growth rate is changing rapidly, the error in such calculations is very large.

The curves in graph 3, excepting extractives (c), are plotted from this table.

TABLE 15

Whole brain: Average percentage increase per day

	3-7 MONTH FETUS	7 MONTH FETUS–1 MONTH	1 MONTH–3 MONTH	3 MONTH–8 MONTH	8 MONTH–21 YEARS
Whole brain	1.25	1.31	0.41	0.067	0.013
Water	1.24	1.28	0.39	0.065	0.014
Solids	1.30	1.47	0.55	0.081	0.01
Phosphatids	1.31	1.59	0.34	0.13	0.012
Cerebrosides	0.00	(2.23)	2.69	0.14	0.016
Sulphatids	1.41	1.16	1.70	0.075	0.018
Cholesterol	1.46	1.63	0.47	0.073	0.018
Total lipins	1.36	1.59	0.62	0.093	0.014
Proteins	1.27	1.42	0.66	0.087	0.007
Organic extractives	1.28	1.52	0.32	0.046	0.0
Inorganic extractives	1.32	1.28	0.25	0.067	0.0
Total extractives	1.30	1.44	0.29	0.048	0.0

Calculated from data in table 11. The average number of milligrams added per day was divided by the average weight for the given period, instead of the weight at the beginning of the period, as is usually done. When there are rapid changes in weight, this method is not as accurate as that used in table 12. It is believed that the temporary rise in growth at about the seventh month of fetal life is due to the method of calculation.

The curve marked extractives (c) in graph 3 was plotted from the above data.

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